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(54) Title: METHOD FOR DIAGNOSING PROSTATE CANCER

(57) Abstract: Objective methods for detecting and diagnosing prostate cancer (PRC) or prostatic intraepithelial neoplasia (PIN) are described herein. In one embodiment, the diagnostic method involves the determining a expression level of PRC -associated gene that discriminate between PRC or PIN and nomal cell. The present invention further provides methods of screening for therapeutic agents useful in the treatment of either or both of PRC and PIN, methods of treating either or both of PRC and PIN and method of vaccinating a subject against either or both of PRC and PIN.

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# DESCRIPTION

## METHOD FOR DIAGNOSING PROSTATE CANCER

The present application is related to USSN 60/414.873, filed September 30, 2002, which is incorporated herein by reference.

## FIELD OF THE INVENTION

The invention relates to methods of diagnosing prostate cancer.

## BACKGROUND OF THE INVENTION

Prostate cancer (PRC) is one of the most common malignancies in men and represents a significant worldwide health problem. It is the second most frequent cause of cancer death in the United States (1). Incidence of PRC is increasing steadily in developed countries according to the prevalence of Western–style diet and increasing number of senior population. Increasing number of patients also die from this disease in Japan due to adoption of a Western life style (2). Currently, the diagnosis of PRC is based on an increased level of the serum prostate specific antigen (PSA). Early diagnosis provides an opportunity for curative surgery. Patients with organ confined PRC are usually treated and approximately 70% of them are curable with radical prostatectomy (3, 4). Most of patients with advanced or relapsed disease are treated with androgen ablation therapy because growth of PRC is initially androgen dependent. Although most of these patients initially respond to androgen ablation therapy, the disease eventually progresses to androgen-independent PRC, at which point the tumor is no longer responsive to androgen ablation therapy.

One of the most serious clinical problems of treatment for PRC is that this androgen-independent PRC is unresponsive to any other therapies, and understanding the mechanism of androgen-independent growth and establishing new therapies other than androgen ablation therapy against PRC are urgent issues for management of PRC.

On the other hand, prostatic intraepithelial neoplasia (PIN) is the specific type of minimal lesion that is believed to be the precursor of PRC (McNeal & Bostwick, 1986). PIN is regarded as a continuum between low- grade and high-grade forms, and high-grade PIN is considered to be the immediate precursor of invasive carcinoma. High-grade PIN

and PRC frequently coexist and they share the similar chromosomal and genetic alterations (Qian et al., 1999). However, the mechanism of PIN development and the progression from PIN to PRC remain unclear. Therefore, genome-wide analysis of expression profiles in PINs is an essential step toward understanding the molecular carcinogenesis and progression and the preventive strategies of PRC.

cDNA microarray technologies have enabled to obtain comprehensive profiles of gene expression in normal and malignant cells, and compare the gene expression in malignant and corresponding normal cells (Okabe et al., Cancer Res 61:2129-37 (2001); Kitahara et al., Cancer Res 61: 3544-9 (2001); Lin et al., Oncogene 21:4120-8 (2002); Hasegawa et al., Cancer Res 62:7012-7 (2002)). This approach enables to disclose the complex nature of cancer cells, and helps to understand the mechanism of carcinogenesis. Identification of genes that are deregulated in tumors can lead to more precise and accurate diagnosis of individual cancers, and to develop novel therapeutic targets (Bienz and Clevers, Cell 103:311-20 (2000)). To disclose mechanisms underlying tumors from a genome-wide point of view, and discover target molecules for diagnosis and development of novel therapeutic drugs, the present inventors have been analyzing the expression profiles of tumor cells using a cDNA microarray of 23040 genes (Okabe et al., Cancer Res 61:2129-37 (2001); Kitahara et al., Cancer Res 61:3544-9 (2001); Lin et al., Oncogene 21:4120-8 (2002); Hasegawa et al., Cancer Res 62:7012-7 (2002)).

Studies designed to reveal mechanisms of carcinogenesis have already facilitated identification of molecular targets for anti-tumor agents. For example, inhibitors of farnexyltransferase (FTIs) which were originally developed to inhibit the growth-signaling pathway related to Ras, whose activation depends on posttranslational farnesylation, has been effective in treating Ras-dependent tumors in animal models (He et al., Cell 99:335-45 (1999)). Clinical trials on human using a combination or anti-cancer drugs and anti-HER2 monoclonal antibody, trastuzumab, have been conducted to antagonize the proto-oncogene receptor HER2/neu; and have been achieving improved clinical response and overall survival of breast-cancer patients (Lin et al., Cancer Res 61:6345-9 (2001)). A tyrosine kinase inhibitor, STI-571, which selectively inactivates bcr-abl fusion proteins, has been developed to treat chronic myelogenous leukemias wherein constitutive activation of bcr-abl tyrosine kinase plays a crucial role in the transformation of leukocytes. Agents of these kinds are designed to suppress oncogenic activity of specific gene products (Fujita

et al., Cancer Res 61:7722-6 (2001)). Therefore, gene products commonly up-regulated in cancerous cells may serve as potential targets for developing novel anti-cancer agents.

It has been demonstrated that CD8+ cytotoxic T lymphocytes (CTLs) recognize epitope peptides derived from tumor-associated antigens (TAAs) presented on MHC Class I molecule, and lyse tumor cells. Since the discovery of MAGE family as the first example of TAAs, many other TAAs have been discovered using immunological approaches (Boon, Int J Cancer 54: 177-80 (1993); Boon and van der Bruggen, J Exp Med 183: 725-9 (1996); van der Bruggen et al., Science 254: 1643-7 (1991); Brichard et al., J Exp Med 178: 489-95 (1993); Kawakami et al., J Exp Med 180: 347-52 (1994)). Some of the discovered TAAs are now in the stage of clinical development as targets of immunotherapy. TAAs discovered so far include MAGE (van der Bruggen et al., Science 254: 1643-7 (1991)), gp100 (Kawakami et al., J Exp Med 180: 347-52 (1994)), SART (Shichijo et al., J Exp Med 187: 277-88 (1998)), and NY-ESO-1 (Chen et al., Proc Natl Acad Sci USA 94: 1914-8 (1997)). On the other hand, gene products which had been demonstrated to be specifically overexpressed in tumor cells, have been shown to be recognized as targets inducing cellular immune responses. Such gene products include p53 (Umano et al., Brit J Cancer 84: 1052-7 (2001)), HER2/neu (Tanaka et al., Brit J Cancer 84: 94-9 (2001)), CEA (Nukaya et al., Int J Cancer 80: 92-7 (1999)), and so on.

In spite of significant progress in basic and clinical research concerning TAAs (Rosenbeg et al., Nature Med 4: 321-7 (1998); Mukherji et al., Proc Natl Acad Sci USA 92: 8078-82 (1995); Hu et al., Cancer Res 56: 2479-83 (1996)), only limited number of candidate TAAs for the treatment of adenocarcinomas, including colorectal cancer, are available. TAAs abundantly expressed in cancer cells, and at the same time which expression is restricted to cancer cells would be promising candidates as immunotherapeutic targets. Further, identification of new TAAs inducing potent and specific antitumor immune responses is expected to encourage clinical use of peptide vaccination strategy in various types of cancer (Boon and can der Bruggen, J Exp Med 183: 725-9 (1996); van der Bruggen et al., Science 254: 1643-7 (1991); Brichard et al., J Exp Med 178: 489-95 (1993); Kawakami et al.,J Exp Med 180: 347-52 (1994); Shichijo et al., J Exp Med 187: 277-88 (1998); Chen et al., Proc Natl Acad Sci USA 94: 1914-8 (1997); Harris, J Natl Cancer Inst 88: 1442-5 (1996); Butterfield et al., Cancer Res 59: 3134-42 (1999); Vissers et al., Cancer Res 59: 5554-9 (1999); van der Burg et al., J

Immunol 156: 3308-14 (1996); Tanaka et al., Cancer Res 57: 4465-8 (1997); Fujie et al., Int J Cancer 80: 169-72 (1999); Kikuchi et al., Int J Cancer 81: 459-66 (1999); Oiso et al., Int J Cancer 81: 387-94 (1999)).

It has been repeatedly reported that peptide-stimulated peripheral blood mononuclear cells (PBMCs) from certain healthy donors produce significant levels of IFN-γ in response to the peptide, but rarely exert cytotoxicity against tumor cells in an HLA-A24 or –A0201 restricted manner in $^{51}$Cr-release assays (Kawano et al., Cance Res 60: 3550-8 (2000); Nishizaka et al., Cancer Res 60: 4830-7 (2000); Tamura et al., Jpn J Cancer Res 92: 762-7 (2001)). However, both of HLA-A24 and HLA-A0201 are one of the popular HLA alleles in Japanese, as well as Caucasian (Date et al., Tissue Antigens 47: 93-101 (1996); Kondo et al., J Immunol 155: 4307-12 (1995); Kubo et al., J Immunol 152: 3913-24 (1994); Imanishi et al., Proceeding of the eleventh International Hictocompatibility Workshop and Conference Oxford University Press, Oxford, 1065 (1992); Williams et al., Tissue Antigen 49: 129 (1997)). Thus, antigenic peptides of carcinomas presented by these HLAs may be especially useful for the treatment of carcinomas among Japanese and Caucasian. Further, it is known that the induction of low-affinity CTL in vitro usually results from the use of peptide at a high concentration, generating a high level of specific peptide/MHC complexes on antigen presenting cells (APCs), which will effectively activate these CTL (Alexander-Miller et al., Proc Natl Acad Sci USA 93: 4102-7 (1996)).

## SUMMARY OF THE INVENTION

The invention is based on the discovery of a pattern of gene expression correlated with PRC or PIN. The genes that are differentially expressed in either or both of PRC and PIN are collectively referred to herein as "PRC nucleic acids" or "PRC polynucleotides" and the corresponding encoded polypeptides are referred to as "PRC polypeptides" or "PRC proteins."

Accordingly, the invention features a method of diagnosing or determining a predisposition to either or both of PRC and PIN in a subject by determining an expression level of a PRC-associated gene in a patient derived biological sample, such as tissue sample. By PRC associated gene is meant a gene that is characterized by an expression

level which differs in a cell obtained from a PRC or PIN cell compared to a normal cell. A normal cell is one obtained from prostate tissue. A PRC-associated gene includes for example PRC 1-692. An alteration, *e.g.*, increase or decrease of the level of expression of the gene compared to a normal control level of the gene indicates that the subject suffers from or is at risk of developing either or both of PRC and PIN.

By normal control level is meant a level of gene expression detected in a normal, healthy individual or in a population of individuals known not to be suffering from PRC and PIN. A control level is a single expression pattern derived from a single reference population or from a plurality of expression patterns. For example, the control level can be a database of expression patterns from previously tested cells. A normal individual is one with no clinical symptoms of PRC and PIN.

An increase in the level of PRC 1-88,296-321,458-537 detected in a test sample compared to a normal control level indicates the subject (from which the sample was obtained) suffers from or is at risk of developing at least either of PRC or PIN. In contrast, a decrease in the level of PRC 89-295,322-457,538-692 detected in a test sample compared to a normal control level indicates said subject suffers from or is at risk of developing either or both of PRC and PIN.

Alternatively, expression of a panel of PRC-associated genes in the sample is compared to a PRC control level of the same panel of genes. By PRC control level is meant the expression profile of the PRC-associated genes found in a population suffering from either or both of PRC and PIN.

Gene expression is increased or decreased 10%, 25%, 50% compared to the control level. Alternately, gene expression is increased or decreased 1, 2, 5 or more fold compared to the control level. Expression is determined by detecting hybridization, *e.g.*, on an array, of a PRC-associated gene probe to a gene transcript of the patient-derived tissue sample.

The patient derived tissue sample is any tissue from a test subject, e.g., a patient known to or suspected of having PRC or PIN. For example, the tissue contains an epithelial cell. For example, the tissue is an epithelial cell from prostate tissue.

The invention also provides a PRC reference expression profile of a gene expression level of two or more of PRC 1-692. Alternatively, the invention provides a PRC reference expression profile of the levels of expression two or more of PRC 1-88,

PRC 89-295, PRC 296-321, PRC 322-457, PRC 458-537,or PRC 538-692.

The invention further provides methods of identifying an agent that inhibits or enhances the expression or activity of a PRC-associated gene,e.g PRC 1-692 by contacting a test cell expressing a PRC associated gene with a test agent and determining the expression level of the PRC associated gene. The test cell is an epithelial cell such as an epithelial cell from prostate tissue. A decrease of the level compared to a normal control level of the gene indicates that the test agent is an inhibitor of the PRC-associated gene and reduces a symptom of either or both of PRC and PIN. Alternatively, an increase of the level or activity compared to a normal control level or activity of the gene indicates that said test agent is an enhancer of expression or function of the PRC associated gene and reduces a symptom of either or both of PRC and PIN, e.g, PRC 89-295, PRC 322-457, PRC 538-692.

The invention also provides a kit with a detection reagent which binds to two or more PRC nucleic acid sequences or which binds to a gene product encoded by the nucleic acid sequences. Also provided is an array of nucleic acids that binds to two or more PRC nucleic acids.

Therapeutic methods include a method of treating or preventing either or both of PRC and PIN in a subject by administering to the subject an antisense composition. The antisense composition reduces the expression of a specific target gene, e.g., the antisense composition contains a nucleotide, which is complementary to a sequence selected from the group consisting of PRC 1-88, 296-321, 458-537. Another method includes the steps of administering to a subject an small interfering RNA (siRNA) composition. The siRNA composition reduces the expression of a nucleic acid selected from the group consisting of PRC 1-88, 296-321, 458-537. In yet another method, treatment or prevention of either or both of PRC and PIN in a subject is carried out by administering to a subject a ribozyme composition. The nucleic acid-specific ribozyme composition reduces the expression of a nucleic acid selected from the group consisting of PRC 1-88, 296-321, 458-537. Other therapeutic methods include those in which a subject is administered a compound that increases the expression of PRC 89-295, 322-457, 538-692 or activity of a polypeptide encoded by PRC 89-295,322-457,538-692. Furthermore, either or both of PRC and PIN can be treated by administering a protein encoded by PRC 89-295,322-457,538-692. The protein may be directly administered to the patient or, alternatively, may be expressed in

vivo subsequent to being introduced into the patient, for example, by administering an expression vector or host cell carrying the down-regulated marker gene of interest. Suitable mechanisms for in vivo expression of a gene of interest are known in the art.

The invention also includes vaccines and vaccination methods. For example, a method of treating or preventing either or both of PRC and PIN in a subject is carried out by administering to the subject a vaccine containing a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-88, 296-321, 458-537 or an immunologically active fragment such a polypeptide. An immunologically active fragment is a polypeptide that is shorter in length than the full-length naturally-occurring protein and which induces an immune response. For example, an immunologically active fragment at least 8 residues in length and stimulates an immune cell such as a T cell or a B cell. Immune cell stimulation is measured by detecting cell proliferation, elaboration of cytokines (e.g., IL-2), or production of an antibody.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

One advantage of the methods described herein is that the disease is identified prior to detection of overt clinical symptoms. Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

## BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a photograph of a DNA agarose gel showing expression of representative 5 genes and β-actin examined by semi-quantitative RT-PCR using cDNA prepared from amplified RNA. Gene symbols are noted. T and N indicate tujors and normal, respectively for each of 8 patients.

## DETAILED DESCRIPTION

The present invention is based in part on the discovery of changes in expression patterns of multiple nucleic acid sequences in epithelial cells of patients with PRC or PIN. The differences in gene expression were identified by using a comprehensive cDNA microarray system.

cDNA microarray is a powerful tool for identifying genes that may be applicable for development of novel molecular targets for therapeutic purposes (Ishiguro et al., 2002; Yagyu et al., 2002). Now basic research about PRC are rapidly progressed recently by using genome information and new technologies, but most difficulty in studying human PRCs with histological heterogeneity is the inability to isolate pure samples for molecular analysis. Most of the previous studies have used bulk cancer tissues without eliminating contamination by non-cancerous cells including stroma cells, microvasculature cells, fibromuscular cells, inflammatory cells and other epithelial cells from benign lesions including PINs. However, laser microdissection allows us to overcome this hurdle and enables the precise evaluation of pure cell populations (Emmert-Buck et al., 1996) for PRC and PIN cells. Also, we compared gene expression of cancer cells with their corresponding normal epithelial cells as a control in each case. This procedure prevents individuality of gene expression from effecting on data. This study is the first report about precise expression profiles of PRCs and PINs, coupling with LMM. These data would provide important information on prostatic carcinogenesis and would be greatly useful to identify candidate genes whose products can be targeted for drug design for treatment and prevention of PRC.

The gene-expression profiles of cancer cells from 20 PRCs and 10 PINs were analyzed using cDNA microarray representing 23,040 genes coupled with laser microdissection. By comparing expression patterns between cancer cells from diagnostic PRC patients and normal epithelial cells purely selected with Laser Microdisection, 88 genes were identified as commonly up-regulated in PRC and PIN cells, and 207 genes were identified as being commonly down-regulated in PRC and PIN cells. 26 genes were identified as commonly up-regulated in PRC cells, and 136 genes were identified as being commonly down-regulated in PRC cells. 80 genes were identified as commonly up-regulated in PIN cells and 155 genes were identified as being commonly down-regulated in

PIN cells. In addition, selection was made of candidate molecular markers with the potential of detecting cancer-related proteins in serum or sputum of patients, and discovered some potential targets for development of signal-suppressing strategies in human PRC or PIN.

The differentially expressed genes identified herein are used for diagnostic purposes as markers of PRC or PIN and as gene targets, the expression of which is altered to treat or alleviate a symptom of PRC or PIN.

The genes whose expression levels are modulated (*i.e.*, increased or decreased) in either or both of PRC and PIN patients are summarized in Tables 3-8 and are collectively referred to herein as " PRC-associated genes ","PRC nucleic acids" or "PRC polynucleotides" and the corresponding encoded polypeptides are referred to as "PRC polypeptides" or "PRC proteins." Unless indicated otherwise, "PRC" is meant to refer to any of the sequences disclosed herein. (*e.g.*, PRC 1-692). The genes that have been previously described are presented along with a database accession number.

By measuring expression of the various genes in a sample of cells, PRC and PIN are diagnosed. Similarly, by measuring the expression of these genes in response to various agents, agents for treating either or both of PRC and PIN can be identified.

The invention involves determining (*e.g.*, measuring) the expression of at least one, and up to all the PRC sequences listed in Tables 3-8. Using sequence information provided by the GeneBank™ database entries for the known sequences the PRC associated genes are detected and measured using techniques well known to one of ordinary skill in the art. For example, sequences within the sequence database entries corresponding to PRC sequences, are used to construct probes for detecting PRC RNA sequences in, *e.g.*, northern blot hybridization analyses. Probes include at least 10, 20, 50, 100, 200 nucleotides of a reference sequence. As another example, the sequences can be used to construct primers for specifically amplifying the PRC nucleic acid in, *e.g*, amplification-based detection methods such as reverse-transcription based polymerase chain reaction.

Expression level of one or more of the PRC-associated genes in the test cell population, *e.g.*, a patient derived tissues sample is then compared to expression levels of the some genes in a reference population. The reference cell population includes one or more cells for which the compared parameter is known, *i.e.*, PRC cells or non-PRC cells.

Whether or not a pattern of gene expression in the test cell population compared to the reference cell population indicates PRC or PIN, or a predisposition thereto depends upon the composition of the reference cell population. For example, if the reference cell population is composed of non- PRC cells, a similar gene expression pattern in the test cell population and reference cell population indicates the test cell population is non-PRC. Conversely, if the reference cell population is made up of PRC cells, a similar gene expression profile between the test cell population and the reference cell population that the test cell population includes PRC cells.

A level of expression of a PRC marker gene in a test cell population is considered altered in levels of expression if its expression level varies from the reference cell population by more than 1.0, 1.5, 2.0, 5.0, 10.0 or more fold from the expression level of the corresponding PRC marker gene in the reference cell population.

Differential gene expression between a test cell population and a reference cell population is normalized to a control nucleic acid, *e.g.* a housekeeping gene. For example, a control nucleic acid is one which is known not to differ depending on the PRC or non-PRC state of the cell. Expression levels of the control nucleic acid in the test and reference nucleic acid can be used to normalize signal levels in the compared populations. Control genes include β-actin, glyceraldehyde 3- phosphate dehydrogenase or ribosomal protein P1.

The test cell population is compared to multiple reference cell populations. Each of the multiple reference populations may differ in the known parameter. Thus, a test cell population may be compared to a second reference cell population known to contain, *e.g.*, PRC cells, as well as a second reference population known to contain, *e.g.*, non-PRC cells (normal cells). The test cell is included in a tissue type or cell sample from a subject known to contain, or to be suspected of containing, PRC cells.

The test cell is obtained from a bodily tissue or a bodily fluid, *e.g.*, biological fluid (such as blood, or serum). For example, the test cell is purified from a tissue. Preferably, the test cell population comprises a epithelial cell. The epithelial cell is from tissue known to be or suspected to be cancerous.

Cells in the reference cell population are derived from a tissue type as similar to test cell. Optionally, the refernce cell poulation is a cell line, *e.g.* a PRC cell line (positive control) or a norma non-PRC cell line (negative control). Alternatively, the control cell

population is derived from a database of molecular information derived from cells for which the assayed parameter or condition is known.

The subject is preferably a mammal. The mammal can be, *e.g.*, a human, non-human primate, mouse, rat, dog, cat, horse, or cow.

Expression of the genes disclosed herein is determined at the protein or nucleic acid level using methods known in the art. For example, Northern hybridization analysis using probes which specifically recognize one or more of these nucleic acid sequences can be used to determine gene expression. Alternatively, expression is measured using reverse-transcription-based PCR assays, *e.g.*, using primers specific for the differentially expressed gene sequences. Expression is also determined at the protein level, *i.e.*, by measuring the levels of polypeptides encoded by the gene products described herein, or biological activity thereof. Such methods are well known in the art and include, *e.g.*, immunoassays based on antibodies to proteins encoded by the genes. The biological activity of the proteins encoded by the genes are also well known.

*Diagnosing PRC or PIN*

PRC or PIN is diagnosed by measuring the expression level of one or more PRC nucleic acid sequences from a test population of cells, (*i.e.*, a patient derived biological sample). Preferably, the test cell population comprises an epithelial cell, e.g., a cell obtained from prostate tissue. Gene expression is also measured from blood or other bodily fluids such as urine. Other biological samples can be used for measuring the protein level. For example, the protein level in the blood, or serum derived from subject to be diagnosed can be measured by immunoassay or biological assay.

Expression of one or more of an PRC-associated gene, *e.g.*, PRC 1-692 is determined in the test cell or biological sample and compared to the expression of the normal control level. A normal control level is an expression profile of a PRC-associated gene typically found in a population known not to be suffering from PRC. An increase or a decrease of the level of expression in the patient derived tissue sample of the PRC associated genes indicates that the subject is suffering from or is at risk of developing PRC or PIN. For example, an increase in expression of PRC 1-88, PRC 296-321, PRC 458-537 in the test population compared to the normal control level indicates that the subject is

suffering from or is at risk of developing PRC or PIN. Conversely, a decrease in expression of PRC 89-295, PRC 322-457, PRC 538-692 in the test population compared to the normal control level indicates that the subject is suffering from or is at risk of developing PRC or PIN.

When one or more of the PRC-associated genes are altered in the test population compared to the normal control level indicates that the subject suffers from or is at risk of developing PRC or PIN. For example, at least 1%, 5%, 25%, 50%, 60%, 80%, 90% or more of the panel of PRC-associated genes (PRC 1-88, PRC 296-321, PRC 458-537, PRC 89-295, PRC 322-457,or PRC 538-692) are altered.

The expression levels of the PRC 1-692 in a particular specimen can be estimated by quantifying mRNA corresponding to or protein encoded by PRC 1-692. Quantification methods for mRNA are known to those skilled in the art. For example, the levels of mRNAs corresponding to the PRC 1-692 can be estimated by Northern blotting or RT-PCR. Since the nucleotide sequence of the PRC 1-692 have already been reported. Anyone skilled in the art can design the nucleotide sequences for probes or primers to quantify the PRC 1-692.

Also the expression level of the PRC 1-692 can be analyzed based on the activity or quantity of protein encoded by the gene. A method for determining the quantity of the PRC 1-692 protein is shown in bellow. For example, immunoassay method is useful for the determination of the proteins in biological materials. Any biological materials can be used for the determination of the protein or it's activity. For example, blood sample is analyzed for estimation of the protein encoded by a serum marker. On the other hand, a suitable method can be selected for the determination of the activity of a protein encoded by the PRC 1-692 according to the activity of each protein to be analyzed.

In the present invention, a diagnostic agent for diagnosing PRC or PIN, is also provided. The diagnostic agent of the present invention comprises a compound that binds to a polynucleotide or a polypeptide of the present invention. Preferably, an oligonucleotide that hybridizes to the polynucleotide of the PRC 1-692, or an antibody that binds to the polypeptide of the PRC 1-692 may be used as such a compound.

In the present invention, PRC 1-692 are useful for diagnosing either or both of PRC and PIN. PRC 1-295 are useful for diagnosing both of PRC and PIN. PRC 296-457 are also useful for diagnosing PRC as PRC specific markers. Furthermore, PRC 458-692 are

useful for diagnosing PIN as PIN specific markers.

*Identifying Agents that inhibit or enhance PRC-associated gene expression*

An agent that inhibits the expression or activity of an PRC-associated gene is identified by contacting a test cell population expressing an PRC associated upregulated gene with a test agent and determining the expression level of the PRC associated gene. A decrease in expression in the presence of the agent compared to the normal control level (or compared to the level in the absence of the test agent) indicates the agent is an inhibitor of an PRC associated upregulated gene and useful to inhibit PRC or PIN.

Alternatively, an agent that enhances the expression or activity of an PRC downregulated associated gene is identified by contacting a test cell population expressing an PRC associated gene with a test agent and determining the expression level or activity of the PRC associated downregulated gene. An increase of expression or activity compared to a normal control expression level or activity of the PRC-associated gene indicates that the test agent augments expression or activity of the downregulated PRC associated gene.

The test cell population is any cell expressing the PRC-associated genes. For example, the test cell population contains an epithelial cell, such as a cell is or derived from prostate. For example, the test cell is immortalized cell line derived from a PRC cell. Alternatively, the test cell is a cell, which has been transfected with a PRC-associated gene or which has been transfected with a regulatory sequence (*e.g.* promoter sequence) from a PRC-associated gene operably linked to a reporter gene.

*Assessing efficacy of treatment of PRC or PIN in a subject*

The differentially expressed PRC-associated gene identified herein also allow for the course of treatment of either or both of PRC and PIN to be monitored. In this method, a test cell population is provided from a subject undergoing treatment for PRC or PIN. If desired, test cell populations are obtained from the subject at various time points before, during, or after treatment. Expression of one or more of the PRC-associated gene, in the cell population is then determined and compared to a reference cell population which includes cells whose PRC state is known. The reference cells have not been exposed to the treatment.

If the reference cell population contains no PRC cells, a similarity in expression between PRC-associated gene in the test cell population and the reference cell population indicates that the treatment is efficacious. However, a difference in expression between PRC -associated gene in the test population and a normal control reference cell population indicates the a less favorable clinical outcome or prognosis.

By "efficacious" is meant that the treatment leads to a reduction in expression of a pathologically upregulated gene, increase in expression of a pathologically down-regulated gene or a decrease in size, prevalence, or metastatic potential of PRC in a subject. When treatment is applied prophylactically, "efficacious" means that the treatment retards or prevents a PRC or PIN from forming or retards, prevents, or alleviates a symptom of clinical PRC or PIN. Assesment of prostate tumors are made using standard clinical protocols.

Efficaciousness is determined in association with any known method for diagnosing or treating either or both of PRC and PIN. PRC is diagnosed for example, by identifying symptomatic anomalies, *e.g.,* urinary symptoms such as difficulty in starting or stopping the stream, dysuria, frequency, or hematuria.

*Selecting a therapeutic agent for treating PRC or PIN that is appropriate for a particular individual*

Differences in the genetic makeup of individuals can result in differences in their relative abilities to metabolize various drugs. An agent that is metabolized in a subject to act as an inhibitor of PRC or PIN can manifest itself by inducing a change in gene expression pattern in the subject's cells from that characteristic of an PRC state to a gene expression pattern characteristic of a non-PRC state. Accordingly, the differentially expressed PRC-associated gene disclosed herein allow for a putative therapeutic or prophylactic inhibitor of PRC or PIN to be tested in a test cell population from a selected subject in order to determine if the agent is a suitable PRC or PIN inhibitor in the subject.

To identify a inhibitor of PRC or PIN, that is appropriate for a specific subject, a test cell population from the subject is exposed to a therapeutic agent, and the expression of one or more of PRC 1-692 genes is determined.

The test cell population contains a PRC or PIN cell expressing a PRC associated gene. Preferably, the test cell is an epithelial cell. For example a test cell population is incubated in the presence of a candidate agent and the pattern of gene expression of the test

sample is measured and compared to one or more reference profiles, *e.g.*, an PRC reference expression profile or an non-PRC reference expression profile.

A decrease in expression of one or more of PRC 1-88, PRC 296-321, PRC 458-537 or an increase in expression of one or more of PRC 89-295, PRC 322-457, PRC 538-692 in a test cell population relative to a reference cell population containing PRC is indicative that the agent is therapeutic.

The test agent can be any compound or composition. For example, the test agents are immunomodulatory agents.

*Screening assays for identifying therapeutic agents*

The differentially expressed genes disclosed herein can also be used to identify candidate therapeutic agents for treating PRC or PIN. The method is based on screening a candidate therapeutic agent to determine if it converts an expression profile of PRC 1-692 characteristic of an PRC state to a pattern indicative of a non-PRC state.

In the present invention, PRC 1-692 are useful for screening of therapeutic agent for treating or preventing either or both of PRC and PIN. PRC 1-295 are used for screening of therapeutic agent for treating or preventing both of PRC and PIN. PRC 296-457 are also used as PRC specific markers for screening of therapeutic agent for treating or preventing PRC. Furthermore, PRC 458-692 are used as PIN specific markers for screening of therapeutic agent for treating or preventing PIN or preventing PRC.

In the method, a cell is exposed to a test agent or a combination of test agents (sequentially or consequentially) and the expression of one or more PRC 1-692 in the cell is measured. The expression profile of the PRC-associated gene in the test population is compared to expression level of the PRC-associated gene in a reference cell population that is not exposed to the test agent.

An agent effective in stimulating expression of underexpressed genes, or in suppressing expression of overexpressed genes is deemed to lead to a clinical benefit such compounds are further tested for the ability to prevent PRC in animals or test subjects.

In a further embodiment, the present invention provides methods for screening candidate agents which are potential targets in the treatment or prevention of either or both of PRC and PIN. As discussed in detail above, by controlling the expression levels or activities of marker genes, one can control the onset and progression of either or both of PRC and PIN. Thus, candidate agents, which are potential targets in the treatment or

prevention of either or both of PRC and PIN, can be identified through screenings that use the expression levels and activities of marker genes as indices. In the context of the present invention, such screening may comprise, for example, the following steps:

a) contacting a test compound with a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-692, ;

b) detecting the binding activity between the polypeptide and the test compound; and

c) selecting a compound that binds to the polypeptide

Alternatively, the screening method of the present invention may comprise the following steps:

a) contacting a candidate compound with a cell expressing one or more marker genes, wherein the one or more marker genes is selected from the group consisting of PRC 1-692; and

b) selecting a compound that reduces the expression level of one or more marker genes selected from the group consisting of PRC 1-88, 296-321, 458-537, or elevates the expression level of one or more marker genes selected from the group consisting of PRC 89-295,322-457,538-692.

Cells expressing a marker gene include, for example, cell lines established from PRC; such cells can be used for the above screening of the present invention.

Alternatively, the screening method of the present invention may comprise the following steps:

a) contacting a test compound with a polypeptide encoded by a nucleic acid selected from the group consisting of selected from the group consisting of PRC 1-692;

b) detecting the biological activity of the polypeptide of step (a); and

c) selecting a compound that suppresses the biological activity of the polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-88, 296-321, 458-537 in comparison with the biological activity detected in the absence of the test compound, or enhances the the biological activity of the polypeptide encoded by a nucleic acid selected from the group consisting of PRC 89-295,322-457,538-692 in comparison with the biological activity detected in the absence of the test compound.

A protein required for the screening can be obtained as a recombinant protein using

the nucleotide sequence of the marker gene. Based on the information of the marker gene, one skilled in the art can select any biological activity of the protein as an index for screening and a measurement method based on the selected biological activity.

Alternatively, the screening method of the present invention may comprise the following steps:

a) contacting a candidate compound with a cell into which a vector comprising the transcriptional regulatory region of one or more marker genes and a reporter gene that is expressed under the control of the transcriptional regulatory region has been introduced, wherein the one or more marker genes are selected from the group consisting of PRC 1-692

b) measuring the activity of said reporter gene; and

c) selecting a compound that reduces the expression level of said reporter gene when said marker gene is an up-regulated marker gene selected from the group consisting of PRC 1-88, 296-321, 458-537 as compared to a control, or that enhances the expression level of said reporter gene when said marker gene is a down-regulated marker gene selected from the group consisting of PRC 89-295,322-457,538-692, as compared to a control.

Suitable reporter genes and host cells are well known in the art. The reporter construct required for the screening can be prepared by using the transcriptional regulatory region of a marker gene. When the transcriptional regulatory region of a marker gene has been known to those skilled in the art, a reporter construct can be prepared by using the previous sequence information. When the transcriptional regulatory region of a marker gene remains unidentified, a nucleotide segment containing the transcriptional regulatory region can be isolated from a genome library based on the nucleotide sequence information of the marker gene.

The compound isolated by the screening is a candidate for drugs that inhibit the activity of the protein encoded by marker genes and can be applied to the treatment or prevention of PRC or PIN.

Moreover, compound in which a part of the structure of the compound inhibiting the activity of proteins encoded by marker genes is converted by addition, deletion and/or replacement are also included in the compounds obtainable by the screening method of the present invention.

When administrating the compound isolated by the method of the invention as a pharmaceutical for humans and other mammals, such as mice, rats, guinea-pigs, rabbits, cats, dogs, sheep, pigs, cattle, monkeys, baboons, and chimpanzees, the isolated compound can be directly administered or can be formulated into a dosage form using known pharmaceutical preparation methods. For example, according to the need, the drugs can be taken orally, as sugar-coated tablets, capsules, elixirs and microcapsules, or non-orally, in the form of injections of sterile solutions or suspensions with water or any other pharmaceutically acceptable liquid. For example, the compounds can be mixed with pharmaceutically acceptable carriers or media, specifically, sterilized water, physiological saline, plant-oils, emulsifiers, suspending agents, surfactants, stabilizers, flavoring agents, excipients, vehicles, preservatives, binders, and such, in a unit dose form required for generally accepted drug implementation. The amount of active ingredients in these preparations makes a suitable dosage within the indicated range acquirable.

Examples of additives that can be mixed to tablets and capsules are, binders such as gelatin, corn starch, tragacanth gum and arabic gum; excipients such as crystalline cellulose; swelling agents such as corn starch, gelatin and alginic acid; lubricants such as magnesium stearate; sweeteners such as sucrose, lactose or saccharin; and flavoring agents such as peppermint, Gaultheria adenothrix oil and cherry. When the unit-dose form is a capsule, a liquid carrier, such as an oil, can also be further included in the above ingredients. Sterile composites for injections can be formulated following normal drug implementations using vehicles such as distilled water used for injections.

Physiological saline, glucose, and other isotonic liquids including adjuvants, such as D-sorbitol, D-mannnose, D-mannitol, and sodium chloride, can be used as aqueous solutions for injections. These can be used in conjunction with suitable solubilizers, such as alcohol, specifically ethanol, polyalcohols such as propylene glycol and polyethylene glycol, non-ionic surfactants, such as Polysorbate 80 (TM) and HCO-50.

Sesame oil or Soy-bean oil can be used as a oleaginous liquid and may be used in conjunction with benzyl benzoate or benzyl alcohol as a solubilizer and may be formulated with a buffer, such as phosphate buffer and sodium acetate buffer; a pain-killer, such as procaine hydrochloride; a stabilizer, such as benzyl alcohol and phenol; and an anti-oxidant. The prepared injection may be filled into a suitable ampule.

Methods well known to one skilled in the art may be used to administer the

pharmaceutical composition of the present inevntion to patients, for example as intraarterial, intravenous, or percutaneous injections and also as intranasal, transbronchial, intramuscular or oral administrations. The dosage and method of administration vary according to the body-weight and age of a patient and the administration method; however, one skilled in the art can routinely select a suitable metod of administration. If said compound is encodable by a DNA, the DNA can be inserted into a vector for gene therapy and the vector administered to a patient to perform the therapy. The dosage and method of administration vary according to the body-weight, age, and symptoms of the patient but one skilled in the art can suitably select them.

For example, although the dose of a compound that binds to the protein of the present invention and regulates its activity depends on the symptoms, the dose is about 0.1 mg to about 100 mg per day, preferably about 1.0 mg to about 50 mg per day and more preferably about 1.0 mg to about 20 mg per day, when administered orally to a normal adult (weight 60 kg).

When administering parenterally, in the form of an injection to a normal adult (weight 60 kg), although there are some differences according to the patient, target organ, symptoms and method of administration, it is convenient to intravenously inject a dose of about 0.01 mg to about 30 mg per day, preferably about 0.1 to about 20 mg per day and more preferably about 0.1 to about 10 mg per day. Also, in the case of other animals too, it is possible to administer an amount converted to 60 kgs of body-weight.

*Assessing the prognosis of a subject with PRC or PIN*

Also provided is a method of assessing the prognosis of a subject with PRC or PIN by comparing the expression of one or more PRC-associated gene s in a test cell population to the expression of the genes in a reference cell population derived from patients over a spectrum of disease stages. By comparing gene expression of one or more PRC-associated gene in the test cell population and the reference cell population(s), or by comparing the pattern of gene expression over time in test cell populations derived from the subject, the prognosis of the subject can be assessed.

A decrease in expression of one or more of PRC 89-295, PRC 322-457, PRC 538-692 compared to a normal control or an increase of expression of one or more of PRC 1-88, PRC 296-321, PRC 458-537 compared to a normal control indicates less favorable prognosis. An increase in expression of one or more of PRC 89-295, PRC 322-457, PRC

538-692 indicates a more favorable prognosis, and a decrease in expression of PRC 1-88, PRC 296-321, PRC 458-537 indicates a more favorable prognosis for the subject.

*Kits*

The invention also includes an PRC-detection reagent, e.g., a nucleic acid that specifically binds to or identifies one or more PRC nucleic acids such as oligonucleotide sequences, which are complementary to a portion of an PRC nucleic acid or antibodies which bind to proteins encoded by an PRC nucleic acid. The reagents are packaged together in the form of a kit. The reagents are packaged in separate containers, e.g., a nucleic acid or antibody (either bound to a solid matrix or packaged separately with reagents for binding them to the matrix) , a control reagent (positive and/or negative), and/or a detectable label. Instructions (e.g., written, tape, VCR, CD-ROM, etc.) for carrying out the assay are included in the kit. The assay format of the kit is a Northern hybridization or a sandwich ELISA known in the art.

For example, PRC detection reagent, is immobilized on a solid matrix such as a porous strip to form at least one PRC detection site. The measurement or detection region of the porous strip may include a plurality of sites containing a nucleic acid. A test strip may also contain sites for negative and/or positive controls. Alternatively, control sites are located on a separate strip from the test strip. Optionally, the different detection sites may contain different amounts of immobilized nucleic acids, *i.e.*, a higher amount in the first detection site and lesser amounts in subsequent sites. Upon the addition of test sample, the number of sites displaying a detectable signal provides a quantitative indication of the amount of PRC present in the sample. The detection sites may be configured in any suitably detectable shape and are typically in the shape of a bar or dot spanning the width of a teststrip.

Alternatively, the kit contains a nucleic acid substrate array comprising one or more nucleic acid sequences. The nucleic acids on the array specifically identify one or more nucleic acids represented by PRC 1-692. The expression of 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 40 or 50 or more of the nucleic acids represented by PRC 1-692 are identified by virtue if the level of binding to an array test strip or chip. The substrate array can be on, *e.g.*, a solid substrate, *e.g.*, a "chip" as described in U.S. Patent No.5,744,305.

*Arrays and pluralities*

The invention also includes a nucleic acid substrate array comprising one or more nucleic acid. The nucleic acids on the array specifically corresponds to one or more nucleic acid sequences represented by PRC 1-692. The level expression of 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 40 or 50 or more of the nucleic acids represented by PRC 1-692 are identified by detecting nucleic acid binding to the array.

The invention also includes an isolated plurality (*i.e.,* a mixture if two or more nucleic acids) of nucleic acids. The nucleic acids are in a liquid phase or a solid phase, *e.g.,* immobilized on a solid support such as a nitrocellulose membrane. The plurality includes one or more of the nucleic acids represented by PRC 1-692. In various embodiments, the plurality includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 40 or 50 or more of the nucleic acids represented by PRC 1-692.

*Methods of inhibiting PRC or PIN*

The invention provides a method for treating or alleviating a symptom of PRC or PIN in a subject by decreasing expression or activity of PRC 1-88, PRC 296-321, PRC 458-537 or increasing expression or activity of PRC 89-295, PRC 322-457, PRC 538-692. Therapeutic compounds are administered prophylactically or therapeutically to subject suffering from at risk of (or susceptible to) developing PRC or PIN. Such subjects are identified using standard clinical methods or by detecting an aberrant level of expression or activity of (*e.g.*, PRC 1-692). Therapeutic agents include inhibitors of cell cycle regulation, cell proliferation, and protein kinase activity.

In the present invention, PRC 1-692 are useful for treating or preventing either or both of PRC and PIN as molecular target. PRC 1-295 are useful for treating or preventing both of PRC and PIN. PRC 296-457 are also useful for treating or preventing PRC as molecular target. Furthermore, PRC 458-692 are useful for treating or preventing PIN and ultimately preventing PRC.

The therapeutic method includes increasing the expression, or function, or both of one or more gene products of genes whose expression is decreased ("underexpressed genes") in PRC or PIN cell relative to normal cells of the same tissue type from which the PRC or PIN cells are derived. In these methods, the subject is treated with an effective amount of a compound, which increases the amount of one of more of the underexpressed genes in the subject. Administration can be systemic or local. Therapeutic compounds include a polypeptide product of an underexpressed gene, or a biologically active fragment

thereof a nucleic acid encoding an underexpressed gene and having expression control elements permitting expression in the PRC or PIN cells; for example an agent which increases the level of expression of such gene endogenous to the PRC or PIN cells (i.e., which up-regulates expression of the underexpressed gene or genes). Administration of such compounds counter the effects of aberrantly-under expressed of the gene or genes in the subject's prostate cells and improves the clinical condition of the subject.

The method also includes decreasing the expression, or function, or both, of one or more gene products of genes whose expression is aberrantly increased ("overexpressed gene") in. Expression is inhibited in any of several ways known in the art. For example, expression is inhibited by administering to the subject a nucleic acid that inhibits, or antagonizes, the expression of the overexpressed gene or genes, e.g., an antisense oligonucleotide or small interfering RNA which disrupts expression of the overexpressed gene or genes.

Alternatively, function of one or more gene products of the overexpressed genes is inhibited by administering a compound that binds to or otherwise inhibits the function of the gene products. For example, the compound is an antibody which binds to the overexpressed gene product or gene products.

As noted above, antisense nucleic acids corresponding to the nucleotide sequence of PRC 1-88, 296-321, 458-537 can be used to reduce the expression level of the PRC 1-88, 296-321, 458-537. Antisense nucleic acids corresponding to PRC 1-88, 296-321, 458-537 that are up-regulated in either or both of PRC and PIN are useful for the treatment of either or both of PRC and PIN. Specifically, the antisense nucleic acids of the present invention may act by binding to the PRC 1-88, 296-321, 458-537 or mRNAs corresponding thereto, thereby inhibiting the transcription or translation of the genes, promoting the degradation of the mRNAs, and/or inhibiting the expression of proteins encoded by a nucleic acid selected from the group consisting of the PRC 1-88, 296-321, 458-537, finally inhibiting the function of the proteins . The term "antisense nucleic acids" as used herein encompasses both nucleotides that are entirely complementary to the target sequence and those having a mismatch of one or more nucleotides, so long as the antisense nucleic acids can specifically hybridize to the target sequences. For example, the antisense nucleic acids of the present invention include polynucleotides that have a homology of at least 70% or higher, preferably at 80% or higher, more preferably 90% or higher, even more preferably

95% or higher over a span of at least 15 continuous nucleotides. Algorithms known in the art can be used to determine the homology.

The antisense nucleic acid derivatives of the present invention act on cells producing the proteins encoded by marker genes by binding to the DNAs or mRNAs encoding the proteins, inhibiting their transcription or translation, promoting the degradation of the mRNAs, and inhibiting the expression of the proteins, thereby resulting in the inhibition of the protein function.

An antisense nucleic acid derivative of the present invention can be made into an external preparation, such as a liniment or a poultice, by mixing with a suitable base material which is inactive against the derivative.

Also, as needed, the derivatives can be formulated into tablets, powders, granules, capsules, liposome capsules, injections, solutions, nose-drops and freeze-drying agents by adding excipients, isotonic agents, solubilizers, stabilizers, preservatives, pain-killers, and such. These can be prepared by following known methods.

The antisense nucleic acids derivative is given to the patient by directly applying onto the ailing site or by injecting into a blood vessel so that it will reach the site of ailment. An antisense-mounting medium can also be used to increase durability and membrane-permeability. Examples are, liposomes, poly-L-lysine, lipids, cholesterol, lipofectin or derivatives of these.

The dosage of the antisense nucleic acid derivative of the present invention can be adjusted suitably according to the patient's condition and used in desired amounts. For example, a dose range of 0.1 to 100 mg/kg, preferably 0.1 to 50 mg/kg can be administered.

The antisense nucleic acids of the invention inhibit the expression of the protein of the invention and is thereby useful for suppressing the biological activity of a protein of the invention. Also, expression-inhibitors, comprising the antisense nucleic acids of the invention, are useful since they can inhibit the biological activity of a protein of the invention.

The antisense nucleic acids of present invention include modified oligonucleotides. For example, thioated nucleotides may be used to confer nuclease resistance to an oligonucleotide.

Also, a siRNA against marker gene can be used to reduce the expression level of the marker gene. By the term "siRNA" is meant a double stranded RNA molecule which

prevents translation of a target mRNA. Standard techniques of introducing siRNA into the cell are used, including those in which DNA is a template from which RNA is transcribed. In the context of the present invention, the siRNA comprises a sense nucleic acid sequence and an anti-sense nucleic acid sequence against an upregulated marker gene, such as PRC 1-88, 296-321, 458-537. The siRNA is constructed such that a single transcript has both the sense and complementary antisense sequences from the target gene, e.g., a hairpin.

The method is used to alter the expression in a cell of an upregulated, e.g., as a result of malignant transformation of the cells. Binding of the siRNA to a transcript corresponding to one of the PRC 1-88, 296-321, 458-537 in the target cell results in a reduction in the protein production by the cell. The length of the oligonucleotide is at least 10 nucleotides and may be as long as the naturally-occurring the transcript. Preferably, the oligonucleotide is 19-25 nucleotides in length. Most preferably, the oligonucleotide is less than 75, 50 , 25 nucleotides in length.

The nucleotide sequence of the siRNAs were designed using a siRNA design computer program available from the Ambion website (http://www.ambion.com/techlib/misc/siRNA_finder.html). The computer program selects nucleotide sequences for siRNA synthesis based on the following protocol.

Selection of siRNA Target Sites:

1. Beginning with the AUG start codon of the object transcript, scan downstream for AA dinucleotide sequences. Record the occurrence of each AA and the 3' adjacent 19 nucleotides as potential siRNA target sites. Tuschl, et al. recommend against designing siRNA to the 5' and 3' untranslated regions (UTRs) and regions near the start codon (within 75 bases) as these may be richer in regulatory protein binding sites. UTR-binding proteins and/or translation initiation complexes may interfere with the binding of the siRNA endonuclease complex.
2. Compare the potential target sites to the human genome database and eliminate from consideration any target sequences with significant homology to other coding sequences. The homology search can be performed using BLAST, which can be found on the NCBI server at: www.ncbi.nlm.nih.gov/BLAST/
3. Select qualifying target sequences for synthesis. At Ambion, preferably several target sequences can be selected along the length of the gene for evaluation

The antisense oligonucleotide or siRNA of the invention inhibit the expression of the polypeptide of the invention and is thereby useful for suppressing the biological activity of the polypeptide of the invention. Also, expression-inhibitors, comprising the antisense oligonucleotide or siRNA of the invention, are useful in the point that they can inhibit the biological activity of the polypeptide of the invention. Therefore, a composition comprising the antisense oligonucleotide or siRNA of the present invention are useful in treating a PRC or PIN.

Alternatively, function of one or more gene products of the over-expressed genes is inhibited by administering a compound that binds to or otherwise inhibits the function of the gene products. For example, the compound is an antibody which binds to the over-expressed gene product or gene products.

The present invention refers to the use of antibodies, particularly antibodies against a protein encoded by an up-regulated marker gene, or a fragment of the antibody. As used herein, the term "antibody" refers to an immunoglobulin molecule having a specific structure, that interacts (i.e., binds) only with the antigen that was used for synthesizing the antibody (i.e., the up-regulated marker gene product) or with an antigen closely related to it. Furthermore, an antibody may be a fragment of an antibody or a modified antibody, so long as it binds to one or more of the proteins encoded by the marker genes. For instance, the antibody fragment may be Fab, $F(ab')_2$, Fv, or single chain Fv (scFv), in which Fv fragments from H and L chains are ligated by an appropriate linker (Huston J. S. et al. Proc. Natl. Acad. Sci. U.S.A. 85:5879-5883 (1988)). More specifically, an antibody fragment may be generated by treating an antibody with an enzyme, such as papain or pepsin. Alternatively, a gene encoding the antibody fragment may be constructed, inserted into an expression vector, and expressed in an appropriate host cell (see, for example, Co M. S. et al. J. Immunol. 152:2968-2976 (1994); Better M. and Horwitz A. H. Methods Enzymol. 178:476-496 (1989); Pluckthun A. and Skerra A. Methods Enzymol. 178:497-515 (1989); Lamoyi E. Methods Enzymol. 121:652-663 (1986); Rousseaux J. et al. Methods Enzymol. 121:663-669 (1986); Bird R. E. and Walker B. W. Trends Biotechnol. 9:132-137 (1991)).

An antibody may be modified by conjugation with a variety of molecules, such as polyethylene glycol (PEG). The present invention provides such modified antibodies. The modified antibody can be obtained by chemically modifying an antibody. These modification methods are conventional in the field.

Alternatively, an antibody may be obtained as a chimeric antibody, between a variable region derived from a nonhuman antibody and a constant region derived from a human antibody, or as a humanized antibody, comprising the complementarity determining region (CDR) derived from a nonhuman antibody, the frame work region (FR) derived from a human antibody, and the constant region. Such antibodies can be prepared by using known technologies.

Cancer therapies directed at specific molecular alterations that occur in cancer cells have been validated through clinical development and regulatory approval of anti-cancer drugs such as trastuzumab (Herceptin) for the treatment of advanced breast cancer, imatinib methylate (Gleevec) for chronic myeloid leukemia, gefitinib (Iressa) for non-small cell lung cancer (NSCLC), and rituximab (anti-CD20 mAb) for B-cell lymphoma and mantle cell lymphoma (Ciardiello F, Tortora G. A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. Clin Cancer Res. 2001 Oct;7(10):2958-70. Review.; Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med. 2001 Mar 15;344(11):783-92.; Rehwald U, Schulz H, Reiser M, Sieber M, Staak JO, Morschhauser F, Driessen C, Rudiger T, Muller-Hermelink K, Diehl V, Engert A. Treatment of relapsed CD20+ Hodgkin lymphoma with the monoclonal antibody rituximab is effective and well tolerated: results of a phase 2 trial of the German Hodgkin Lymphoma Study Group. Blood. 2003 Jan 15;101(2):420-424.; Fang G, Kim CN, Perkins CL, Ramadevi N, Winton E, Wittmann S and Bhalla KN. (2000). Blood, 96, 2246-2253.). These drugs are clinically effective and better tolerated than traditional anti-cancer agents because they target only transformed cells. Hence, such drugs not only improve survival and quality of life for cancer patients, but also validate the concept of molecularly targeted cancer therapy. Furthermore, targeted drugs can enhance the efficacy of standard chemotherapy when used in combination with it (Gianni L. (2002). Oncology, 63 Suppl 1, 47-56.; Klejman A, Rushen L, Morrione A, Slupianek A and Skorski T. (2002). Oncogene, 21, 5868-5876.). Therefore, future cancer treatments will probably involve combining conventional drugs with target-specific agents aimed at different characteristics of tumor cells such as angiogenesis and invasiveness.

These modulatory methods are performed *ex vivo* or *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). The method involves administering a protein or combination of proteins or a nucleic acid molecule or combination of nucleic acid, molecules as therapy to counteract aberrant expression or activity of the differentially expressed genes.

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity of the genes may be treated with therapeutics that antagonize (*i.e.*, reduce or inhibit) activity of the overexpressed gene or genes. Therapeutics that antagonize activity are administered therapeutically or prophylactically.

Therapeutics that may be utilized include, *e.g.*, (*i*) a polypeptide, or analogs, derivatives, fragments or homologs thereof of the underexpressed gene or genes; (*ii*) antibodies to the overexpressed gene or genes; (*iii*) nucleic acids encoding the underexpressed gene or genes; (*iv*) antisense nucleic acids or nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the coding sequences of one or more overexpressed genes); (*v*) small interfering RNA (siRNA); or (*vi*) modulators (*i.e.*, inhibitors, agonists and antagonists that alter the interaction between an over/underexpressed polypeptide and its binding partner. The dysfunctional antisense molecules are utilized to "knockout" endogenous function of a polypeptide by homologous recombination (see, *e.g.*, Capecchi, *Science* 244: 1288-1292 1989)

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with therapeutics that increase (*i.e.*, are agonists to) activity. Therapeutics that upregulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, a polypeptide (or analogs, derivatives, fragments or homologs thereof) or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of a gene whose expression is altered). Methods that are well-known within the art include, but are not limited to, immunoassays (*e.g.*, by Western blot analysis, immunoprecipitation

followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (*e.g.,* Northern assays, dot blots, *in situ* hybridization, etc.).

Prophylactic administration occurs prior to the manifestation of overt clinical symptoms of disease, such that a disease or disorder is prevented or, alternatively, delayed in its progression.

Therapeutic methods include contacting a cell with an agent that modulates one or more of the activities of the gene products of the differentially expressed genes. An agent that modulates protein activity includes a nucleic acid or a protein, a naturally-occurring cognate ligand of these proteins, a peptide, a peptidomimetic, or other small molecule. For example, the agent stimulates one or more protein activities of one or more of a differentially under-expressed gene.

The present invention also relates to a method of treating or preventing either or both of PRC and PIN in a subject comprising administering to said subject a vaccine comprising a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-88, 296-321, 458-537 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide or the fragment thereof. An administration of the polypeptide induce an anti-tumor immunity in a subject. To inducing anti-tumor immunity, a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-88, 296-321, 458-537 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide is administered. The polypeptide or the immunologically active fragments thereof are useful as vaccines against either or both of PRC and PIN. In some cases the proteins or fragments thereof may be administered in a form bound to the T cell recepor (TCR) or presented by an antigen presenting cell (APC), such as macrophage, dendritic cell (DC), or B-cells. Due to the strong antigen presenting ability of DC, the use of DC is most preferable among the APCs.

In the present invention, vaccine against either or both of PRC and PIN refers to a substance that has the function to induce anti-tumor immunity upon inoculation into animals. According to the present invention, polypeptides encoded by a nucleic acid selected from the group consisting of PRC 1-88, 296-321, 458-537 or fragments thereof were suggested to be HLA-A24 or HLA-A*0201 restricted epitopes peptides that may induce potent and specific immune response against either or both of PRC and PIN cells

expressing PRC 1-88, 296-321, 458-537. Thus, the present invention also encompasses method of inducing anti-tumor immunity using the polypeptides. In general, anti-tumor immunity includes immune responses such as follows:

- induction of cytotoxic lymphocytes against tumors,
- induction of antibodies that recognize tumors, and
- induction of anti-tumor cytokine production.

Therefore, when a certain protein induces any one of these immune responses upon inoculation into an animal, the protein is decided to have anti-tumor immunity inducing effect. The induction of the anti-tumor immunity by a protein can be detected by observing in vivo or in vitro the response of the immune system in the host against the protein.

For example, a method for detecting the induction of cytotoxic T lymphocytes is well known. A foreign substance that enters the living body is presented to T cells and B cells by the action of antigen presenting cells (APCs). T cells that respond to the antigen presented by APC in antigen specific manner differentiate into cytotoxic T cells (or cytotoxic T lymphocytes; CTLs) due to stimulation by the antigen, and then proliferate (this is referred to as activation of T cells). Therefore, CTL induction by a certain peptide can be evaluated by presenting the peptide to T cell by APC, and detecting the induction of CTL. Furthermore, APC has the effect of activating CD4+ T cells, CD8+ T cells, macrophages, eosinophils, and NK cells. Since CD4+ T cells and CD8+ T cells are also important in anti-tumor immunity, the anti-tumor immunity inducing action of the peptide can be evaluated using the activation effect of these cells as indicators.

A method for evaluating the inducing action of CTL using dendritic cells (DCs) as APC is well known in the art. DC is a representative APC having the strongest CTL inducing action among APCs. In this method, the test polypeptide is initially contacted with DC, and then this DC is contacted with T cells. Detection of T cells having cytotoxic effects against the cells of interest after the contact with DC shows that the test polypeptide has an activity of inducing the cytotoxic T cells. Activity of CTL against tumors can be detected, for example, using the lysis of $^{51}$Cr-labeled tumor cells as the indicator. Alternatively, the method of evaluating the degree of tumor cell damage using $^{3}$H-thymidine uptake activity or LDH (lactose dehydrogenase)-release as the indicator is also well known.

Apart from DC, peripheral blood mononuclear cells (PBMCs) may also be used as the APC. The induction of CTL is reported that the it can be enhanced by culturing PBMC in the presence of GM-CSF and IL-4. Similarly, CTL has been shown to be induced by culturing PBMC in the presence of keyhole limpet hemocyanin (KLH) and IL-7.

The test polypeptides confirmed to possess CTL inducing activity by these methods are polypeptides having DC activation effect and subsequent CTL inducing activity. Therefore, polypeptides that induce CTL against tumor cells are useful as vaccines against tumors. Furthermore, APC that acquired the ability to induce CTL against tumors by contacting with the polypeptides are useful as vaccines against tumors. Furthermore, CTL that acquired cytotoxicity due to presentation of the polypeptide antigens by APC can be also used as vaccines against tumors. Such therapeutic methods for tumors using anti-tumor immunity due to APC and CTL are referred to as cellular immunotherapy.

Generally, when using a polypeptide for cellular immunotherapy, efficiency of the CTL-induction is known to increase by combining a plurality of polypeptides having different structures and contacting them with DC. Therefore, when stimulating DC with protein fragments, it is advantageous to use a mixture of multiple types of fragments.

Alternatively, the induction of anti-tumor immunity by a polypeptide can be confirmed by observing the induction of antibody production against tumors. For example, when antibodies against a polypeptide are induced in a laboratory animal immunized with the polypeptide, and when growth of tumor cells is suppressed by those antibodies, the polypeptide can be determined to have an ability to induce anti-tumor immunity.

Anti-tumor immunity is induced by administering the vaccine of this invention, and the induction of anti-tumor immunity enables treatment and prevention of either or both of PRC and PIN. Therapy against cancer or prevention of the onset of cancer includes any of the steps, such as inhibition of the growth of cancerous cells, involution of cancer, and suppression of occurrence of cancer. Decrease in mortality of individuals having cancer, decrease of tumor markers in the blood, alleviation of detectable symptoms accompanying cancer, and such are also included in the therapy or prevention of cancer. Such therapeutic and preventive effects are preferably statistically significant. For example, in observation, at a significance level of 5% or less, wherein the therapeutic or preventive effect of a vaccine against cell proliferative diseases is compared to a control

without vaccine administration. For example, Student's t-test, the Mann-Whitney U-test, or ANOVA may be used for statistical analyses.

The above-mentioned protein having immunological activity or a vector encoding the protein may be combined with an adjuvant. An adjuvant refers to a compound that enhances the immune response against the protein when administered together (or successively) with the protein having immunological activity. Examples of adjuvants include cholera toxin, salmonella toxin, alum, and such, but are not limited thereto. Furthermore, the vaccine of this invention may be combined appropriately with a pharmaceutically acceptable carrier. Examples of such carriers are sterilized water, physiological saline, phosphate buffer, culture fluid, and such. Furthermore, the vaccine may contain as necessary, stabilizers, suspensions, preservatives, surfactants, and such. The vaccine is administered systemically or locally. Vaccine administration may be performed by single administration, or boosted by multiple administrations.

When using APC or CTL as the vaccine of this invention, tumors can be treated or prevented, for example, by the ex vivo method. More specifically, PBMCs of the subject receiving treatment or prevention are collected, the cells are contacted with the polypeptide ex vivo, and following the induction of APC or CTL, the cells may be administered to the subject. APC can be also induced by introducing a vector encoding the polypeptide into PBMCs ex vivo. APC or CTL induced in vitro can be cloned prior to administration. By cloning and growing cells having high activity of damaging target cells, cellular immunotherapy can be performed more effectively. Furthermore, APC and CTL isolated in this manner may be used for cellular immunotherapy not only against individuals from whom the cells are derived, but also against similar types of tumors from other individuals.

Furthermore, a pharmaceutical composition for treating or preventing a cell proliferative disease, such as cancer, comprising a pharmaceutically effective amount of the polypeptide of the present invention is provided. The pharmaceutical composition may be used for raising anti tumor immunity.

*Pharmaceutical compositions for inhibiting PRC or PIN*

Pharmaceutical formulations include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration, or for administration by inhalation or

insufflation. Preferably, administration is intravenous. The formulations are optionally packaged in discrete dosage units

Pharmaceutical formulations suitable for oral administration include capsules, cachets or tablets, each containing a predetermined amount of the active ingredient. Formulations also include powders, granules or solutions, suspensions or emulsions. The active ingredient os optionally administered as a bolus electuary or paste. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrant or wetting agents. A tablet may be made by compression or molding, optionally with one or more formulational ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be coated according to methods well known in the art. Oral fluid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives. The tablets may optionally be formulated so as to provide slow or controlled release of the active ingredient therein. A package of tablets may contain one tablet to be taken on each day of the month.

Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline, water-for-injection, immediately prior to use. Alternatively, the formulations may be presented for continuous infusion. Extemporaneous injection solutions and suspensions

may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations for rectal administration include suppositories with standard carriers such as cocoa butter or polyethylene glycol. Formulations for topical administration in the mouth, for example buccally or sublingually, include lozenges, which contain the active ingredient in a flavored base such as sucrose and acacia or tragacanth, and pastilles comprising the active ingredient in a base such as gelatin and glycerin or sucrose and acacia. For intra-nasal administration the compounds of the invention may be used as a liquid spray or dispersible powder or in the form of drops. Drops may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilizing agents or suspending agents.

For administration by inhalation the compounds are conveniently delivered from an insufflator, nebulizer, pressurized packs or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichiorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation or insufflation, the compounds may take the form of a dry powder composition, for example a powder mix of the compound and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form, in for example, capsules, cartridges, gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insufflators.

Other formulations include implantable devices and adhesive patches; which release a therapeutic agent.

When desired, the above described formulations, adapted to give sustained release of the active ingredient, may be employed. The pharmaceutical compositions may also contain other active ingredients such as antimicrobial agents, immunosuppressants or preservatives.

It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include flavoring agents.

Preferred unit dosage formulations are those containing an effective dose, as recited below, or an appropriate fraction thereof, of the active ingredient.

For each of the aforementioned conditions, the compositions, e.g., polypeptides and organic compounds are administered orally or via injection at a dose of from about 0.1 to about 250 mg/kg per day. The dose range for adult humans is generally from about 5 mg to about 17.5 g/day, preferably about 5 mg to about 10 g/day, and most preferably about 100 mg to about 3 g/day. Tablets or other unit dosage forms of presentation provided in discrete units may conveniently contain an amount which is effective at such dosage or as a multiple of the same, for instance, units containing about 5 mg to about 500 mg, usually from about 100 mg to about 500 mg.

The dose employed will depend upon a number of factors, including the age and sex of the subject, the precise disorder being treated, and its severity. Also the route of administration may vary depending upon the condition and its severity.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims. The following examples illustrate the identification and characterization of genes differentially expressed in PRC or PIN cells.

## EXAMPLE 1: PREPARATION OF TEST SAMPLES

Tissue obtained from diseased tissue (e.g., epithelial cells from PRCs) and normal tissues was evaluated to identify genes which are differently expressed or a disease state, *e.g.,* PRC. The assays were carried out as follows.

Patients, tissue samples and Laser-capture microdissection (LCM)

PRC samples including non-cancerous prostate tissues were obtained from 26 patients who underwent radical prostatectomy without preoperative treatment. Prostate adenocarcinomas or high-grade PINs were histopathologically diagnosed by a single pathologist (M.F.). Among 26 PRC tissues, 20 cancers and 10 high-grade PINs cells that have sufficient amount and quality of RNA to analyze were used for microarray study. Clinical and pathological information on the tumor is detailed in Table 1. Samples were embedded in TissueTek OCT medium (Sakura) and then stored at -80°C until use. Frozen specimens were serially sectioned in 8-μm slices with a cryostat and

stained with hematoxylin and eosin to define the analyzed regions. To avoid cross-contamination of cancer and noncancerous cells, the two populations were prepared by EZ Cut LCM System (SL Microtest GmbH) following the manufacture's protocol with several modifications.

Table 1 Clinicopathological features

| Case | Age | PSA (ng/ml) | Pathological Stage | Microdissected Lesions |
|---|---|---|---|---|
| 1 | 76 | 17.0 | pT2aN0M0 | T[(a)] |
| 2 | 73 | 14.0 | pT2aN0M0 | T |
| 3 | 73 | 59.2 | pT3aN0M0 | T |
| 4 | 56 | 8.6 | pT2bN0M0 | T |
| 5 | 73 | 1.8 | pT2aN0M0 | PIN |
| 6 | 61 | 8.9 | pT2bN0M0 | T |
| 7 | 71 | 11.4 | pT2bN0M0 | T |
| 8 | 69 | 9.5 | pT2aN0M0 | PIN |
| 9 | 66 | 9.6 | pT3aN0M0 | T |
| 10 | 62 | 6.7 | pT2aN0M0 | PIN |
| 11 | 56 | 35.0 | pT3bN0M0 | PIN |
| 12 | 66 | 12.0 | pT2bN0M0 | T |
| 13 | 65 | 4.1 | pT2bN0M0 | T |
| 14 | 77 | 12.3 | pT2bN0M0 | T, PIN |
| 15 | 69 | 10.4 | pT2bN0M0 | T |
| 16 | 68 | 14.1 | pT3aN0M0 | T, PIN |
| 17 | NA[(b)] | 10.5 | pT2bN0M0 | T |
| 18 | NA | NA | NA | T |
| 19 | 63 | 4.5 | pT2bN0M0 | T |
| 20 | 67 | 9.8 | pT3aN0M0 | T |
| 21 | 63 | 12.4 | pT3N0M0 | T |
| 22 | 73 | 13.0 | pT3bN1M0 | T |
| 23 | 75 | 10.0 | pT2aN1M0 | T, PIN |
| 24 | 67 | 3.3 | pT3aN0M0 | T, PIN |
| 25 | 64 | 5.7 | pT2bN0M0 | PIN |
| 26 | 69 | 38.0 | pT3aN0M0 | PIN |

[(a)]T indicates prostate cancer. [(b)]NA: not available

Extraction of RNA and T7-based RNA amplification

Total RNA was extracted from each population of laser captured cells into 350μl RLT lysis buffer (QIAGEN). The extracted RNA was treated for 30 minutes at

room temperature with 30 units of DNase I (QIAGEN) in the presence of 1 unit of RNase inhibitor (TOYOBO, Osaka, Japan) to eliminate any contaminating genomic DNA. After inactivation at 70°C for 10 min, the RNAs were purified with an RNeasy Mini Kit (QIAGEN) according to the manufacturer's recommendations and DNase-treated RNAs were subjected to T7-based RNA amplification. Two rounds of amplification yielded 50-100 μg of amplified RNA (aRNA) for each sample. 2.5μg aliquots of aRNA from each cancerous cell and noncancerous cell were reverse-transcribed in the presence of Cy5-dCTP and Cy3-dCTP, respectively.

Preparation of the cDNA microarray

A "genome-wide" cDNA microarray system was prepared containing 23,040 cDNAs selected from the UniGene database o (build #131) the National Center for Biotechnology Information (NCBI). Briefly, the cDNAs were amplified by reverse transcription-PCR using poly(A)+RNA isolated from various human organs as templates; lengths of the amplicons ranged from 200 to 1100 bp without repetitive or poly(A) sequences. The PCR products were spotted in duplicate on type-7 glass slides (Amersham Bioscience) using an Array Spotter Generation III (Amersham Bioscience). Each slide contained 52 housekeeping genes, to normalize the signal intensities of the different fluorescent dyes.

Hybridization and acquisition of data

Hybridization and washing were performed according to protocols described previously except that all processes were carried out with an Automated Slide Processor (Amersham Biosciences) (17). The intensity of each hybridization signal was calculated photometrically by the ArrayVision computer program (Amersham Biosciences) and background intensity was subtracted. Normalization of each Cy3- and Cy5 signal intensity was performed using averaged signals from the 52 housekeeping genes. A cut-off value for each expression level was automatically calculated according to background fluctuation. When both Cy3 and Cy5 signal intensities were lower than the cut-off values, expression of the corresponding gene in that sample was assessed as absent. The Cy5/Cy3 ratio was calculated as the relative expression ratio. For other genes we calculated the Cy5/Cy3 ratio using raw data of each sample.

**EXAMPLE 2 : IDENTIFICATION OF PRC – ASSOCIATED GENES**

When up- or down-regulated genes common to PRC and PINs were identified, the genes were analyzed by the following criteria. Initially, genes whose relative expression ratio was able to be calculated for more than 50% cases and whose expression were up- or down-regulated in more than 50% of cases were selected. The relative expression ratio of each gene (Cy5/Cy3 intensity ratio) was classified into one of four categories: (1) up-regulated (expression ratio more than 3.0 in more than 50% of the informative; (2) down-regulated (expression ratio less than 0.33 in more than 50% of the informative cases; (3) unchanged expression (expression ratio between 0.33 and 3.0 in more than 50% of the informative cases); and (4) not expressed (or slight expression but under the cut-off level for detection). These categories were defined to detect a set of genes whose changes in expression ratios were common among samples as well as specific to a certain subgroup. To detect candidate genes that were commonly up- or down-regulated in either or both of PRC and PIN cell, the overall expression patterns of 23,040 genes were screened to select genes with expression ratios of more than 3.0 or less than 0.33 that were present in more than 50% of the PRC cases categorized as (1), (2), or (3).

Furthermore when up- or down-regulated genes common to PRC or PINs were identified, the genes were analyzed by the following criteria. Initially, genes whose relative expression ratio was able to be calculated for more than 50% cases and whose expression were up- or down-regulated in more than 50% of cases were selected. The relative expression ratio of each gene (Cy5/Cy3 intensity ratio) was classified into one of four categories: (5) up-regulated (expression ratio more than 5.0 in more than 50% of the informative; (6) down-regulated (expression ratio less than 0.2 in more than 50% of the informative cases; (7) unchanged expression (expression ratio between 0.2 and 5.0 in more than 50% of the informative cases); and (8) not expressed (or slight expression but under the cut-off level for detection). These categories were defined to detect a set of genes whose changes in expression ratios were common among samples as well as specific to a certain subgroup. To detect candidate genes that were commonly up- or down-regulated in either or both of PRC and PIN cell, the overall expression patterns of 23,040 genes were screened to select genes with expression ratios of more than 5.0 or less than 0.2 that were present in more than 50% of the PRC cases categorized as (5), (6), or (7).

Identification of genes with clinically relevant expression patterns in PRC cells

The expression patterns of approximately 23,000 genes were investigated in PRC cells using cDNA microarray. Individual data was excluded when both Cy5 and Cy3 signals were under cut-off values. 88 up-regulated genes were identified whose expression ratio was more than 3.0 in PRC and PINs(*see* Table 3), whereas 207 down-regulated genes whose expression ratio was less than 0.33 were identified (*see* Table 4). 26 up-regulated genes were identified whose expression ratio was more than 5.0 in PRC (*see* Table 5), whereas 136 down-regulated genes whose expression ratio was less than 0.2 were identified (*see* Table 6).

Among the up-regulated genes, α-methylacyl coenzyme A racemase (AMACR) has been already reported to be overexpressed in PRC (13). Furthermore, these up-regulated elements included significant genes involved in metabolism and signal transduction pathway, transcriptional factors, cell cycle, oncogene, and cell adhesion and cytoskeleton. Of them, olfactory receptor, family 51, subfamily E, member 2 (*OR51E2*) that is prostate specific G-protein coupled receptor (*PSGR*), and PRC overexpressed gene 1 (*POV1*) had already been reported as over-expressed in PRCs (Luo et al., 2002; Cole et al., 1998; Xu et al., 2000) (*see* Table 5).

80 up-regulated genes were identified whose expression ratio was more than 5.0 in PINs(*see* Table 7), whereas 155 down-regulated genes whose expression ratio was less than 0.2 were identified (*see* Table 8).

To confirm the reliability of the expression indicated by microarray analysis, semi-quantitative RT-PCR experiments were performed. Four up-regulated genes were selected and their expression levels measured by semi-quantitative RT-PCR. A 3-μg aliquot of aRNA from each sample was reverse-transcribed for single-stranded cDNAs using random primer (Roche) and Superscript II (Life Technologies, Inc.). Each cDNA mixture was diluted for subsequent PCR amplification with the primer sets that were shown in Table 2. Expression of β-actin (ACTB) served as an internal control. PCR reactions were optimized for the number of cycles to ensure product intensity within the linear phase of amplification.

Comparing the ratios of the expression levels of the 4 up-regulated genes (AMACR, HOXC6, POV1, ABHD2 andC20ORF102) whose expression were overexpressed in almost of all informative cases the results were highly similar to those of

the microarray analysis in the great majority of the tested cases (Fig. 1). These data verified the reliability of our strategy to identify commonly up-regulated genes in PRC cells.

Table 3 Commonly up-regulated genes in prostate cancers and PINs

| PRC Assignment | Accession No. | Hs. | Symbol | Title |
|---|---|---|---|---|
| | **function known** | | | |
| 1 | M93107 | 76893 | BDH | 3-hydroxybutyrate dehydrogenase (heart, mitochondrial) |
| 2 | U89281 | 11958 | RODH | 3-hydroxysteroid epimerase |
| 3 | L41559 | 3192 | PCBD | 6-pyruvoyl-tetrahydropterin synthase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1) |
| 4 | AJ130733 | 128749 | AMACR | alpha-methylacyl-CoA racemase |
| 5 | S77410 | 89472 | AGTR1 | angiotensin II receptor, type 1 |
| 6 | AI080640 | 413945 | AGR2 | anterior gradient 2 homolog (Xenepus laevis) |
| 7 | NM_000487 | 88251 | ARSA | arylsulfatase A |
| 8 | AF071202 | 139336 | ABCC4 | ATP-binding cassette, sub-family C (CFTR/MRP), member 4 |
| 9 | NM_000060 | 78885 | BTD | biotinidase |
| 10 | D90276 | 12 | CEACAM4 | carcinoembryonic antigen-related cell adhesion molecule 4 |
| 11 | AB030905 | 406384 | CBX3 | chromobox homolog 3 (HP1 gamma homolog, Drosophila) |
| 12 | BF106962 | 20415 | FAM3B | chromosome 21 open reading frame 11 |
| 13 | AI817172 | 29423 | COLEC12 | collectin sub-family member 12 |
| 14 | NM_005436 | 288862 | D10S170 | DNA segment on chromosome 10 (unique) 170 |
| 15 | U31556 | 2331 | E2F5 | E2F transcription factor 5, p130-binding |
| 16 | AF039918 | 80975 | ENTPD5 | ectonucleoside triphosphate diphosphohydrolase 5 |
| 17 | L10340 | 2642 | EEF1A2 | eukaryotic translation elongation factor 1 alpha 2 |
| 18 | AI984005 | 380785 | XPOT | exportin, tRNA (nuclear export receptor |

| | | | | |
|---|---|---|---|---|
| | | | | for tRNAs) |
| 19 | NM_000166 | 333303 | GJB1 | gap junction protein, beta 1, 32kDa (connexin 32, Charcot-Marie-Tooth neuropathy, X-linked) |
| 20 | AF040260 | 105435 | GMDS | GDP-mannose 4,6-dehydratase |
| 21 | AF236056 | 182793 | GOLPH2 | golgi phosphoprotein 2 |
| 22 | AF055013 | 203862 | GNAI1 | guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1 |
| 23 | NM_000856 | 75295 | GUCY1A3 | guanylate cyclase 1, soluble, alpha 3 |
| 24 | S82986 | 820 | HOXC6 | homeo box C6 |
| 25 | U42408 | 18141 | LAD1 | ladinin 1 |
| 26 | M88468 | 130607 | MVK | mevalonate kinase (mevalonic aciduria) |
| 27 | D56064 | 167 | MAP2 | microtubule-associated protein 2 |
| 28 | AI302799 | 68583 | MIPEP | mitochondrial intermediate peptidase |
| 29 | AB002387 | 118483 | MYO6 | myosin VI |
| 30 | R22536 | 220324 | FLJ13052 | NAD kinase |
| 31 | AI246554 | 31547 | NDUFA8 | NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 8, 19kDa |
| 32 | AA858162 | 124673 | NCAG1 | NCAG1 |
| 33 | AI805082 | 303171 | OR51E2 | olfactory receptor, family 51, subfamily E, member 2 |
| 34 | U79240 | 79337 | PASK | PAS domain containing serine/threonine kinase |
| 35 | BF690393 | 83383 | PRDX4 | peroxiredoxin 4 |
| 36 | AK025460 | 286049 | PSA | phosphoserine aminotransferase |
| 37 | NM_021200 | 380812 | PLEKHB1 | pleckstrin homology domain containing, family B (evectins) member 1 |
| 38 | L14778 | 272458 | PPP3CA | protein phosphatase 3 (formerly 2B), catalytic subunit, alpha isoform (calcineurin A alpha) |
| 39 | AF044588 | 344037 | PRC1 | protein regulator of cytokinesis 1 |
| 40 | NM_006765 | 71119 | N33 | Putative prostate cancer tumor suppressor |
| 41 | NM_012342 | 78776 | NMA | putative transmembrane protein |
| 42 | M77836 | 79217 | PYCR1 | pyrroline-5-carboxylate reductase 1 |
| 43 | D42063 | 199179 | RANBP2 | RAN binding protein 2 |
| 44 | AF064824 | 103755 | RIPK2 | receptor-interacting serine-threonine kinase 2 |

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|---|---|---|---|---|
| 45 | N78357 | 302136 | RIMS1 | regulating synaptic membrane exocytosis 1 |
| 46 | L10333 | 99947 | RTN1 | reticulon 1 |
| 47 | Y18418 | 272822 | RUVBL1 | RuvB-like 1 (E. coli) |
| 48 | U80456 | 27311 | SIM2 | single-minded homolog 2 (Drosophila) |
| 49 | AF269150 | 8203 | SMBP | SM-11044 binding protein |
| 50 | U17566 | 84190 | SLC19A1 | solute carrier family 19 (folate transporter), member 1 |
| 51 | D88308 | 11729 | SLC27A2 | solute carrier family 27 (fatty acid transporter), member 2 |
| 52 | AF007216 | 5462 | SLC4A4 | solute carrier family 4, sodium bicarbonate cotransporter, member 4 |
| 53 | AD001528 | 89718 | SMS | spermine synthase |
| 54 | M32313 | 552 | SRD5A1 | steroid-5-alpha-reductase, alpha polypeptide 1 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 1) |
| 55 | L15203 | 82961 | TFF3 | trefoil factor 3 (intestinal) |
| 56 | M91670 | 174070 | E2-EPF | ubiquitin carrier protein |
| 57 | AW135763 | 6375 | HT010 | uncharacterized hypothalamus protein HT010 |
| | **function unknown** | | | |
| 58 | AA206763 | 7991 | C20orf 102 | chromosome 20 open reading frame 102 |
| 59 | AI989530 | 240845 | DKFZP 434D14 6 | DKFZP434D146 protein |
| 60 | AI192351 | 76285 | DKFZP 564B16 7 | DKFZP564B167 protein |
| 61 | AI133467 | 95612 | | ESTs |
| 62 | H17800 | 438858 | | ESTs |
| 63 | AI732103 | | | ESTs |
| 64 | AI671006 | 5794 | | ESTs, Moderately similar to hypothetical protein FLJ20234 [Homo sapiens] [H.sapiens] |
| 65 | AA420675 | 188826 | | ESTs, Moderately similar to RL39_HUMAN 60S ribosomal protein L39 [H.sapiens] |
| 66 | AI700341 | 110406 | | ESTs, Weakly similar to hypothetical protein FLJ20489 [Homo sapiens] [H.sapiens] |

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|---|---|---|---|---|
| 67 | BF057183 | 355809 | | ESTs, Weakly similar to male-specific lethal 3-like 1 isoform |
| 68 | H05758 | 355684 | | ESTs, Weakly similar to neuronal thread protein [Homo sapiens] |
| 69 | AA743348 | 120591 | | Homo sapiens cDNA FLJ35632 fis, clone SPLEN2011678. |
| 70 | AA679304 | 5740 | | Homo sapiens cDNA FLJ40165 fis, clone TESTI2015962. |
| 71 | AK027019 | 381105 | | Homo sapiens cDNA: FLJ23366 fis, clone HEP15665. |
| 72 | AA994004 | 128790 | | Homo sapiens mRNA full length insert cDNA clone EUROIMAGE 1628928 |
| 73 | H09779 | 283851 | | Homo sapiens mRNA; cDNA DKFZp547G036 (from clone DKFZp547G036) |
| 74 | BE254330 | 14846 | | Homo sapiens mRNA; cDNA DKFZp564D016 (from clone DKFZp564D016) |
| 75 | AL157505 | 21380 | | Homo sapiens mRNA; cDNA DKFZp586P1124 (from clone DKFZp586P1124) |
| 76 | AI217963 | 434541 | | Homo sapiens, clone IMAGE:4429946, mRNA |
| 77 | BF724600 | 22247 | | Homo sapiens, clone IMAGE:5302158, mRNA |
| 78 | NM_012066 | 128702 | 20D7-FC4 | hypothetical protein 20D7-FC4 |
| 79 | AB029008 | 84045 | FLJ20288 | FLJ20288 protein |
| 80 | AK026325 | 235873 | FLJ22672 | hypothetical protein FLJ22672 |
| 81 | R55332 | 379386 | LOC115286 | hypothetical protein LOC115286 |
| 82 | H12084 | 31110 | MGC34827 | hypothetical protein MGC34827 |
| 83 | D29954 | 13421 | KIAA0056 | KIAA0056 protein |
| 84 | AB020637 | 167115 | KIAA0830 | KIAA0830 protein |
| 85 | AB023157 | 131945 | KIAA0940 | KIAA0940 protein |

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|---|---|---|---|---|
| 86 | AB032981 | 102657 | KIAA1155 | KIAA1155 protein |
| 87 | AB032983 | 21894 | KIAA1157 | KIAA1157 protein |
| 88 | AB033091 | 446390 | KIAA1265 | KIAA1265 protein |

Table 4 Commonly down-regulated genes in prostate cancers and PINs

| PRC Assignment | Accession No. | Hs. | Symbol | Title |
|---|---|---|---|---|
| | **function known** | | | |
| 89 | NM_002526 | 153952 | NT5E | 5'-nucleotidase, ecto (CD73) |
| 90 | NM_005159 | 118127 | ACTC | actin, alpha, cardiac muscle |
| 91 | NM_001615 | 378774 | ACTG2 | actin, gamma 2, smooth muscle, enteric |
| 92 | AL117643 | 99954 | ACVR1B | activin A receptor, type IB |
| 93 | BG105547 | 324470 | ADD3 | adducin 3 (gamma) |
| 94 | AF245505 | 72157 | DKFZp564I1922 | adlican |
| 95 | N74230 | 193228 | AGXT2 | alanine-glyoxylate aminotransferase 2 |
| 96 | K03000 | 76392 | ALDH1A1 | aldehyde dehydrogenase 1 family, member A1 |
| 97 | M28443 | 300280 | AMY2A | amylase, alpha 2A; pancreatic |
| 98 | AF286598 | 9271 | AMOT | angiomotin |
| 99 | NM_000700 | 78225 | ANXA1 | annexin A1 |
| 100 | D00017 | 217493 | ANXA2 | annexin A2 |
| 101 | NM_001155 | 118796 | ANXA6 | annexin A6 |
| 102 | AK027126 | 160786 | ASS | argininosuccinate synthetase |
| 103 | AA054346 | 32168 | AUTS2 | autism susceptibility candidate 2 |
| 104 | AL117565 | 6607 | AXUD1 | AXIN1 up-regulated 1 |
| 105 | AB004066 | 171825 | BHLHB2 | basic helix-loop-helix domain containing, class B, 2 |
| 106 | M14745 | 79241 | BCL2 | B-cell CLL/lymphoma 2 |
| 107 | S67310 | 69771 | BF | B-factor, properdin |
| 108 | M69225 | 198689 | BPAG1 | bullous pemphigoid antigen 1, |

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|---|---|---|---|---|
| | | | | 230/240kDa |
| 109 | X63629 | 2877 | CDH3 | cadherin 3, type 1, P-cadherin (placental) |
| 110 | R45979 | 252387 | CELSR1 | cadherin, EGF LAG seven-pass G-type receptor 1 (flamingo homolog, Drosophila) |
| 111 | AF134640 | 7235 | CACNG3 | calcium channel, voltage-dependent, gamma subunit 3 |
| 112 | D17408 | 21223 | CNN1 | calponin 1, basic, smooth muscle |
| 113 | K01144 | 84298 | CD74 | CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated) |
| 114 | NM_001878 | 183650 | CRABP2 | cellular retinoic acid binding protein 2 |
| 115 | NM_002996 | 80420 | CX3CL1 | chemokine (C-X3-C motif) ligand 1 |
| 116 | U16306 | 81800 | CSPG2 | chondroitin sulfate proteoglycan 2 (versican) |
| 117 | AV648364 | 356416 | CBX7 | chromobox homolog 7 |
| 118 | AF000959 | 110903 | CLDN5 | claudin 5 (transmembrane protein deleted in velocardiofacial syndrome) |
| 119 | NM_001831 | 75106 | CLU | clusterin (SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2) |
| 120 | L02870 | 1640 | COL7A1 | collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive) |
| 121 | AF018081 | 78409 | COL18A1 | collagen, type XVIII, alpha 1 |
| 122 | NM_001733 | 1279 | C1R | complement component 1, r subcomponent |
| 123 | J04080 | 434029 | C1S | complement component 1, s subcomponent |
| 124 | K02765 | 284394 | C3 | complement component 3 |
| 125 | AF007162 | 408767 | CRYAB | crystallin, alpha B |
| 126 | L12579 | 147049 | CUTL1 | cut-like 1, CCAAT displacement protein (Drosophila) |
| 127 | NM_000076 | 106070 | CDKN1C | cyclin-dependent kinase inhibitor 1C (p57, Kip2) |
| 128 | BF183952 | 412999 | CSTA | cystatin A (stefin A) |
| 129 | NM_004078 | 108080 | CSRP1 | cysteine and glycine-rich protein 1 |
| 130 | J04813 | 104117 | CYP3A5 | cytochrome P450, family 3, subfamily A, |

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|---|---|---|---|---|
| | | | | polypeptide 5 |
| 131 | AF070590 | 90869 | LOC90957 | DEAH-box RNA/DNA helicase AAM73547 |
| 132 | NM_004394 | 75189 | DAP | death-associated protein |
| 133 | D83407 | 156007 | DSCR1L1 | Down syndrome critical region gene 1-like 1 |
| 134 | L11329 | 1183 | DUSP2 | dual specificity phosphatase 2 |
| 135 | AW002941 | 339283 | ERAP140 | endoplasmic reticulum associated protein 140 kDa |
| 136 | J04162 | 176663 | FCGR3A | Fc fragment of IgG, low affinity IIIa, receptor for (CD16) |
| 137 | M87770 | 278581 | FGFR2 | fibroblast growth factor receptor 2 |
| 138 | X02761 | 287820 | FN1 | fibronectin 1 |
| 139 | NM_001456 | 195464 | FLNA | filamin A, alpha (actin binding protein 280) |
| 140 | U60115 | 239069 | FHL1 | four and a half LIM domains 1 |
| 141 | L42176 | 8302 | FHL2 | four and a half LIM domains 2 |
| 142 | U28963 | 380901 | GPS2 | G protein pathway suppressor 2 |
| 143 | AW949747 | 169946 | GATA3 | GATA binding protein 3 |
| 144 | AK021685 | 234896 | GMNN | geminin, DNA replication inhibitor |
| 145 | BF115308 | 132760 | G6PT1 | glucose-6-phosphatase, transport (glucose-6-phosphate) protein 1 |
| 146 | NM_002083 | 2704 | GPX2 | glutathione peroxidase 2 (gastrointestinal) |
| 147 | NM_002084 | 386793 | GPX3 | glutathione peroxidase 3 (plasma) |
| 148 | AA290738 | 301961 | GSTM1 | glutathione S-transferase M1 |
| 149 | NM_002081 | 2699 | GPC1 | glypican 1 |
| 150 | M55543 | 171862 | GBP2 | guanylate binding protein 2, interferon-inducible |
| 151 | NM_000186 | 250651 | HF1 | H factor 1 (complement) |
| 152 | AK000415 | 250666 | HES1 | hairy and enhancer of split 1, (Drosophila) |
| 153 | AA522530 | 111244 | RTP801 | HIF-1 responsive RTP801 |
| 154 | AA490691 | 421136 | HOXD11 | homeo box D11 |
| 155 | J02770 | 36602 | IF | I factor (complement) |
| 156 | S81914 | 76095 | IER3 | immediate early response 3 |
| 157 | M87790 | 102950 | IGLJ3 | immunoglobulin lambda joining 3 |

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|---|---|---|---|---|
| 158 | L08488 | 32309 | INPP1 | inositol polyphosphate-1-phosphatase |
| 159 | M31159 | 77326 | IGFBP3 | insulin-like growth factor binding protein 3 |
| 160 | M59911 | 265829 | ITGA3 | integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor) |
| 161 | X52186 | 85266 | ITGB4 | integrin, beta 4 |
| 162 | NM_006435 | 174195 | IFITM2 | interferon induced transmembrane protein 2 (1-8D) |
| 163 | NM_002198 | 80645 | IRF1 | interferon regulatory factor 1 |
| 164 | AF020201 | 166154 | JAG2 | jagged 2 |
| 165 | M25629 | 123107 | KLK1 | kallikrein 1, renal/pancreas/salivary |
| 166 | X14640 | 74070 | KRT13 | keratin 13 |
| 167 | X07696 | 80342 | KRT15 | keratin 15 |
| 168 | NM_000422 | 2785 | KRT17 | keratin 17 |
| 169 | Y00503 | 182265 | KRT19 | keratin 19 |
| 170 | M21389 | 433845 | KRT5 | keratin 5 (epidermolysis bullosa simplex, Dowling-Meara/Kobner/Weber-Cockayne types) |
| 171 | X03212 | 23881 | KRT7 | keratin 7 |
| 172 | NM_000226 | 2783 | KRT9 | keratin 9 (epidermolytic palmoplantar keratoderma) |
| 173 | D14520 | 84728 | KLF5 | Kruppel-like factor 5 (intestinal) |
| 174 | U07643 | 105938 | LTF | lactotransferrin |
| 175 | D37766 | 75517 | LAMB3 | laminin, beta 3 |
| 176 | AW139663 | 166254 | VMP1 | likely ortholog of rat vacuole membrane protein 1 |
| 177 | AF002672 | 152944 | LOH11CR2A | loss of heterozygosity, 11, chromosomal region 2, gene A |
| 178 | AI814306 | 42438 | LSM6 | LSM6 homolog, U6 small nuclear RNA associated (S. cerevisiae) |
| 179 | Z68179 | 77667 | LY6E | lymphocyte antigen 6 complex, locus E |
| 180 | BE621666 | 296398 | LAPTM4B | lysosomal associated protein transmembrane 4 beta |
| 181 | M33906 | 198253 | HLA-DQA1 | major histocompatibility complex, class II, DQ alpha 1 |
| 182 | K01171 | 409805 | HLA-DRA | major histocompatibility complex, class II, DR alpha |
| 183 | M15178 | 318720 | HLA-DRB4 | major histocompatibility complex, class II, DR beta 4 |

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|---|---|---|---|---|
| 184 | BF697545 | 365706 | MGP | matrix Gla protein |
| 185 | AW298180 | 2256 | MMP7 | matrix metalloproteinase 7 (matrilysin, uterine) |
| 186 | AF017418 | 104105 | MEIS2 | Meis1, myeloid ecotropic viral integration site 1 homolog 2 (mouse) |
| 187 | BF971884 | 118786 | MT2A | metallothionein 2A |
| 188 | NM_005928 | 3745 | MFGE8 | milk fat globule-EGF factor 8 protein |
| 189 | J05581 | 89603 | MUC1 | mucin 1, transmembrane |
| 190 | AA628530 | 405873 | ISYNA1 | myo-inositol 1-phosphate synthase A1 |
| 191 | J02854 | 9615 | MYL9 | myosin, light polypeptide 9, regulatory |
| 192 | AF005888 | 173162 | NOC4 | neighbor of COX4 |
| 193 | AA886412 | 69285 | NRP1 | neuropilin 1 |
| 194 | L31881 | 35841 | NFIX | nuclear factor I/X (CCAAT-binding transcription factor) |
| 195 | X75918 | 82120 | NR4A2 | nuclear receptor subfamily 4, group A, member 2 |
| 196 | M13692 | 572 | ORM1 | orosomucoid 1 |
| 197 | U90878 | 75807 | PDLIM1 | PDZ and LIM domain 1 (elfin) |
| 198 | NM_005036 | 998 | PPARA | peroxisome proliferative activated receptor, alpha |
| 199 | AF035959 | 24879 | PPAP2C | phosphatidic acid phosphatase type 2C |
| 200 | AB003723 | 18079 | PIGQ | phosphatidylinositol glycan, class Q |
| 201 | D00244 | 77274 | PLAU | plasminogen activator, urokinase |
| 202 | AF091434 | 43080 | PDGFC | platelet derived growth factor C |
| 203 | AF027208 | 112360 | PROML1 | prominin-like 1 (mouse) |
| 204 | AL045876 | 430637 | PTGDS | prostaglandin D2 synthase 21kDa (brain) |
| 205 | BF510741 | 5648 | PSMD9 | proteasome (prosome, macropain) 26S subunit, non-ATPase, 9 |
| 206 | M65066 | 1519 | PRKAR1B | protein kinase, cAMP-dependent, regulatory, type I, beta |
| 207 | AW249758 | 96593 | ARHF | ras homolog gene family, member F (in filopodia) |
| 208 | X73427 | 75256 | RGS1 | regulator of G-protein signalling 1 |
| 209 | U72066 | 29287 | RBBP8 | retinoblastoma binding protein 8 |
| 210 | NM_003979 | 194691 | RAI3 | retinoic acid induced 3 |
| 211 | L20688 | 83656 | ARHGDIB | Rho GDP dissociation inhibitor (GDI) beta |
| 212 | X64652 | 241567 | RBMS1 | RNA binding motif, single stranded interacting protein 1 |

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|---|---|---|---|---|
| 213 | AA173755 | 301198 | ROBO1 | roundabout, axon guidance receptor, homolog 1 (Drosophila) |
| 214 | AF132734 | 107394 | SEC8 | secretory protein SEC8 |
| 215 | NM_004636 | 82222 | SEMA3B | sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3B |
| 216 | M93056 | 183583 | SERPINB1 | serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1 |
| 217 | M13690 | 151242 | SERPING1 | serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1 |
| 218 | NM_006456 | 288215 | STHM | sialyltransferase |
| 219 | AF070609 | 75379 | SLC1A3 | solute carrier family 1 (glial high affinity glutamate transporter), member 3 |
| 220 | AF215636 | 5944 | SLC11A3 | solute carrier family 11 (proton-coupled divalent metal ion transporters), member 3 |
| 221 | U59299 | 90911 | SLC16A5 | solute carrier family 16 (monocarboxylic acid transporters), member 5 |
| 222 | Y08110 | 101657 | SORL1 | sortilin-related receptor, L(DLR class) A repeats-containing |
| 223 | M81635 | 160483 | STOM | stomatin |
| 224 | U15131 | 79265 | ST5 | suppression of tumorigenicity 5 |
| 225 | BF514189 | 345728 | SOCS3 | suppressor of cytokine signaling 3 |
| 226 | AI423028 | 71622 | SMARCD3 | SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3 |
| 227 | U21847 | 82173 | TIEG | TGFB inducible early growth response |
| 228 | U54831 | 75248 | TOP2B | topoisomerase (DNA) II beta 180kDa |
| 229 | S95936 | 396489 | TF | transferrin |
| 230 | M77349 | 118787 | TGFBI | transforming growth factor, beta-induced, 68kDa |
| 231 | NM_003186 | 433399 | TAGLN | transgelin |
| 232 | M98479 | 75307 | TGM2 | transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase) |
| 233 | L24203 | 82237 | TRIM29 | tripartite motif-containing 29 |
| 234 | AF208860 | 159651 | TNFRSF21 | tumor necrosis factor receptor superfamily, member 21 |
| 235 | U44839 | 171501 | USP11 | ubiquitin specific protease 11 |

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|---|---|---|---|---|
| 236 | L13852 | 16695 | UBE1L | ubiquitin-activating enzyme E1-like |
| 237 | X63187 | 2719 | WFDC2 | WAP four-disulfide core domain 2 |
| 238 | AF122922 | 284122 | WIF1 | WNT inhibitory factor 1 |
| 239 | AA909999 | 50216 | ZFD25 | zinc finger protein (ZFD25) |
| 240 | AA916688 | 85155 | ZFP36L1 | zinc finger protein 36, C3H type-like 1 |
| | **function unknown** | | | |
| 241 | AB002384 | 101359 | C6orf32 | chromosome 6 open reading frame 32 |
| 242 | AA620628 | 186486 | | ESTs |
| 243 | AA632025 | 444752 | | ESTs |
| 244 | AA904658 | 117299 | | ESTs |
| 245 | AI022658 | 292171 | | ESTs |
| 246 | AI027791 | 132296 | | ESTs |
| 247 | AI338011 | 132147 | | ESTs |
| 248 | AI732637 | 277901 | | ESTs |
| 249 | BE868254 | 380149 | | ESTs |
| 250 | H53099 | 420009 | | ESTs |
| 251 | N95414 | 55168 | | ESTs, Weakly similar to neuronal thread protein [Homo sapiens] [H.sapiens] |
| 252 | BG163478 | 405950 | | ESTs, Weakly similar to BAI1_HUMAN Brain-specific angiogenesis inhibitor 1 precursor [H.sapiens] |
| 253 | AI342255 | 24192 | | Homo sapiens cDNA FLJ20767 fis, clone COL06986. |
| 254 | AI651212 | 4283 | | Homo sapiens cDNA FLJ31125 fis, clone IMR322000819. |
| 255 | AW967916 | 31944 | | Homo sapiens cDNA FLJ33236 fis, clone ASTRO2002571. |
| 256 | AI566720 | 380045 | | Homo sapiens cDNA FLJ34528 fis, clone HLUNG2008066 |
| 257 | W93000 | 59389 | | Homo sapiens cDNA FLJ38601 fis, clone HEART2003781. |
| 258 | BE885999 | 397414 | | Homo sapiens cDNA: FLJ20860 fis, clone ADKA01632. |
| 259 | AK025909 | 288741 | | Homo sapiens cDNA: FLJ22256 fis, clone HRC02860. |
| 260 | AK025953 | 380437 | | Homo sapiens cDNA: FLJ22300 fis, clone HRC04759. |
| 261 | BF311166 | 110783 | | Homo sapiens cDNA: FLJ22365 fis, clone HRC06613. |
| 262 | AI097529 | 8136 | | Homo sapiens clone 23698 mRNA sequence |

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|---|---|---|---|---|
| 263 | AI269367 | 101307 | | Homo sapiens HUT11 protein mRNA, partial 3' UTR |
| 264 | N58556 | 323053 | | Homo sapiens mRNA full length insert cDNA clone EUROIMAGE 26539. |
| 265 | AL110236 | 321022 | | Homo sapiens mRNA; cDNA DKFZp566P1124 (from clone DKFZp566P1124) |
| 266 | BF791544 | 351680 | | Homo sapiens, clone IMAGE:4103364, mRNA |
| 267 | AV733210 | 367688 | | Homo sapiens, clone IMAGE:4794726, mRNA |
| 268 | AA456955 | 78026 | | Homo sapiens, Similar to hypothetical protein C130031J23, clone IMAGE:3445545, mRNA, partial cds |
| 269 | AL120399 | 343567 | LOC151568 | hypothetical protein BC009491 |
| 270 | BE539165 | 355793 | DKFZp313M 0720 | hypothetical protein DKFZp313M0720 |
| 271 | AA709155 | 104800 | FLJ10134 | hypothetical protein FLJ10134 |
| 272 | AK001061 | 30925 | FLJ10199 | hypothetical protein FLJ10199 |
| 273 | AK001431 | 5105 | FLJ10569 | hypothetical protein FLJ10569 |
| 274 | AI709055 | 115412 | FLJ13881 | hypothetical protein FLJ13881 |
| 275 | AK026058 | 27556 | FLJ22405 | hypothetical protein FLJ22405 |
| 276 | AW271223 | 5890 | FLJ23306 | hypothetical protein FLJ23306 |
| 277 | N31935 | 220745 | FLJ25604 | hypothetical protein FLJ25604 |
| 278 | AK001839 | 206501 | LOC57228 | hypothetical protein from clone 643 |
| 279 | AK022547 | 8694 | LOC56965 | hypothetical protein from EUROIMAGE 1977056 |
| 280 | AA180145 | 351270 | LOC152485 | hypothetical protein LOC152485 |
| 281 | AK024828 | 69388 | LOC221749 | hypothetical protein LOC221749 |
| 282 | AV758898 | 366 | MGC27165 | hypothetical protein MGC27165 |
| 283 | AW888223 | 59384 | MGC3047 | hypothetical protein MGC3047 |
| 284 | AA133590 | 377830 | MGC44669 | hypothetical protein MGC44669 |
| 285 | L13720 | 207251 | MGC5560 | hypothetical protein MGC5560 |
| 286 | AI206046 | 50535 | MGC7036 | hypothetical protein MGC7036 |
| 287 | AB002319 | 8663 | KIAA0321 | KIAA0321 protein |
| 288 | AB011125 | 105749 | KIAA0553 | KIAA0553 protein |
| 289 | AB037797 | 24684 | KIAA1376 | KIAA1376 protein |
| 290 | AI741882 | 278436 | KIAA1474 | KIAA1474 protein |
| 291 | AA521149 | 17767 | KIAA1554 | KIAA1554 protein |
| 292 | N62352 | 24790 | KIAA1573 | KIAA1573 protein |
| 293 | AW976121 | 301444 | KIAA1673 | KIAA1673 |

| | | | | |
|---|---|---|---|---|
| 294 | AI890497 | 28501 | KIAA1754 | KIAA1754 protein |
| 295 | T78873 | 9587 | KIAA2002 | KIAA2002 protein |

Table 5 Commonly up-regulated genes in 20 prostate cancers

| PRC Assignment | Accession No. | Hs. | Symbol | Title |
|---|---|---|---|---|
| | **function known** | | | |
| 296 | X12433 | 99364 | ABHD2 | abhydrolase domain containing 2 |
| 297 | AF039018 | 135281 | ALP | alpha-actinin-2-associated LIM protein |
| 298 | AJ130733 | 128749 | AMACR | alpha-methylacyl-CoA racemase |
| 299 | J02611 | 75736 | APOD | apolipoprotein D |
| 300 | AF071202 | 139336 | ABCC4 | ATP-binding cassette, sub-family C (CFTR/MRP), member 4 |
| 301 | AA633487 | 108708 | CAMKK2 | calcium/calmodulin-dependent protein kinase kinase 2, beta |
| 302 | AF001436 | 12289 | CDC42EP2 | CDC42 effector protein (Rho GTPase binding) 2 |
| 303 | BF981201 | 408061 | FABP5 | fatty acid binding protein 5 (psoriasis-associated) |
| 304 | D14446 | 107 | FGL1 | fibrinogen-like 1 |
| 305 | S82986 | 820 | HOXC6 | homeo box C6 |
| 306 | AF064493 | 4980 | LDB2 | LIM domain binding 2 |
| 307 | AI767296 | 123655 | NPR3 | natriuretic peptide receptor C/guanylate cyclase C (atrionatriuretic peptide receptor C) |
| 308 | AA858162 | 124673 | NCAG1 | NCAG1 |
| 309 | AI805082 | 303171 | OR51E2 | olfactory receptor, family 51, subfamily E, member 2 (prostate-specific G protein-coupled receptor) |
| 310 | AF045584 | 18910 | POV1 | prostate cancer overexpressed gene 1 |
| 311 | AI298501 | 21192 | SDK1 | sidekick homolog 1 (chicken) |
| 312 | U80456 | 27311 | SIM2 | single-minded homolog 2 (Drosophila) |
| 313 | AD001528 | 89718 | SMS | spermine synthase |
| 314 | N21096 | 99291 | STXBP6 | syntaxin binding protein 6 (amisyn) |
| 315 | L15203 | 82961 | TFF3 | trefoil factor 3 (intestinal) |
| | **function unknown** | | | |
| 316 | D14657 | 81892 | KIAA0101 | KIAA0101 gene product |
| 317 | AI989530 | 240845 | DKFZP434D146 | DKFZP434D146 protein |

| | | | | |
|---|---|---|---|---|
| 318 | NM_012066 | 128702 | 20D7-FC4 | hypothetical protein 20D7-FC4 |
| 319 | AA206763 | 7991 | C20orf102 | chromosome 20 open reading frame 102 |
| 320 | AI700341 | 110406 | | ESTs, Weakly similar to hypothetical protein FLJ20489 [Homo sapiens] |
| 321 | AI003798 | 23799 | | Homo sapiens, clone IMAGE:4791783, mRNA |

Table 6 Commonly down-regulated genes in 20 prostate cancers

| PRC Assignment | Accession No. | Hs. | Symbol | Title |
|---|---|---|---|---|
| | **function known** | | | |
| 322 | AI827230 | 374481 | APCDD1 | adenomatosis polyposis coli down-regulated 1 |
| 323 | BF965257 | 74120 | APM2 | adipose specific 2 |
| 324 | AF245505 | 72157 | DKFZp564I1922 | adlican |
| 325 | D00017 | 217493 | ANXA2 | annexin A2 |
| 326 | NM_001155 | 118796 | ANXA6 | annexin A6 |
| 327 | AK027126 | 160786 | ASS | argininosuccinate synthetase |
| 328 | W91908 | 6079 | GALNAC4S-6ST | B cell RAG associated protein |
| 329 | AB004066 | 171825 | BHLHB2 | basic helix-loop-helix domain containing, class B, 2 |
| 330 | M14745 | 79241 | BCL2 | B-cell CLL/lymphoma 2 |
| 331 | S67310 | 69771 | BF | B-factor, properdin |
| 332 | M69225 | 198689 | BPAG1 | bullous pemphigoid antigen 1, 230/240kDa |
| 333 | X63629 | 2877 | CDH3 | cadherin 3, type 1, P-cadherin (placental) |
| 334 | AF134640 | 7235 | CACNG3 | calcium channel, voltage-dependent, gamma subunit 3 |
| 335 | M94345 | 82422 | CAPG | capping protein (actin filament), gelsolin-like |
| 336 | AF035752 | 139851 | CAV2 | caveolin 2 |
| 337 | K01144 | 84298 | CD74 | CD74 antigen |
| 338 | AI750036 | 22116 | CDC14B | CDC14 cell division cycle 14 homolog B (S. cerevisiae) |
| 339 | NM_00299 | 80420 | CX3CL1 | chemokine (C-X3-C motif) ligand 1 |

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|---|---|---|---|---|
| | 6 | | | |
| 340 | AF000959 | 110903 | CLDN5 | claudin 5 (transmembrane protein deleted in velocardiofacial syndrome) |
| 341 | NM_00183 1 | 75106 | CLU | clusterin (SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2) |
| 342 | NM_00173 3 | 1279 | C1R | complement component 1, r subcomponent |
| 343 | K02765 | 284394 | C3 | complement component 3 |
| 344 | D13639 | 75586 | CCND2 | cyclin D2 |
| 345 | BF183952 | 412999 | CSTA | cystatin A (stefin A) |
| 346 | M62401 | 82568 | CYP27A1 | cytochrome P450, family 27, subfamily A, polypeptide 1 |
| 347 | J04813 | 104117 | CYP3A5 | cytochrome P450, family 3, subfamily A, polypeptide 5 |
| 348 | X90579 | 166079 | CYP3A5P2 | cytochrome P450, family 3, subfamily A, polypeptide 5 pseudogene 2 |
| 349 | AW956111 | 79404 | D4S234E | DNA segment on chromosome 4 (unique) 234 expressed sequence |
| 350 | AB012955 | 129867 | KIP2 | DNA-dependent protein kinase catalytic subunit-interacting protein 2 |
| 351 | D83407 | 156007 | DSCR1L1 | Down syndrome critical region gene 1-like 1 |
| 352 | L11329 | 1183 | DUSP2 | dual specificity phosphatase 2 |
| 353 | NM_00142 1 | 151139 | ELF4 | E74-like factor 4 (ets domain transcription factor) |
| 354 | AW300770 | 61265 | FAM3D | family with sequence similarity 3, member D |
| 355 | D84239 | 111732 | FCGBP | Fc fragment of IgG binding protein |
| 356 | AF182316 | 234680 | FER1L3 | fer-1-like 3, myoferlin (C. elegans) |
| 357 | M87770 | 278581 | FGFR2 | fibroblast growth factor receptor 2 |
| 358 | NM_00145 6 | 195464 | FLNA | filamin A, alpha (actin binding protein 280) |
| 359 | L42176 | 8302 | FHL2 | four and a half LIM domains 2 |
| 360 | NM_00016 5 | 74471 | GJA1 | gap junction protein, alpha 1, 43kDa (connexin 43) |
| 361 | AW949747 | 169946 | GATA3 | GATA binding protein 3 |
| 362 | NM_00208 3 | 2704 | GPX2 | glutathione peroxidase 2 (gastrointestinal) |
| 363 | NM_00208 4 | 386793 | GPX3 | glutathione peroxidase 3 (plasma) |

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|---|---|---|---|---|
| 364 | AA290738 | 301961 | GSTM1 | glutathione S-transferase M1 |
| 365 | NM_002081 | 2699 | GPC1 | glypican 1 |
| 366 | M55543 | 171862 | GBP2 | guanylate binding protein 2, interferon-inducible |
| 367 | AA666119 | 92287 | GBP3 | guanylate binding protein 3 |
| 368 | NM_000186 | 250651 | HF1 | H factor 1 (complement) |
| 369 | AA490691 | 421136 | HOXD11 | homeo box D11 |
| 370 | J02770 | 36602 | IF | I factor (complement) |
| 371 | S81914 | 76095 | IER3 | immediate early response 3 |
| 372 | AV646610 | 34853 | ID4 | inhibitor of DNA binding 4, dominant negative helix-loop-helix protein |
| 373 | L08488 | 32309 | INPP1 | inositol polyphosphate-1-phosphatase |
| 374 | M31159 | 77326 | IGFBP3 | insulin-like growth factor binding protein 3 |
| 375 | M59911 | 265829 | ITGA3 | integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor) |
| 376 | X52186 | 85266 | ITGB4 | integrin, beta 4 |
| 377 | AF020201 | 166154 | JAG2 | jagged 2 |
| 378 | X14640 | 74070 | KRT13 | keratin 13 |
| 379 | X07696 | 80342 | KRT15 | keratin 15 |
| 380 | M21389 | 433845 | KRT5 | keratin 5 (epidermolysis bullosa simplex, Dowling-Meara/Kobner/Weber-Cockayne types) |
| 381 | X03212 | 23881 | KRT7 | keratin 7 |
| 382 | AF287272 | 84728 | KLF5 | Kruppel-like factor 5 (intestinal) |
| 383 | Y00711 | 234489 | LDHB | lactate dehydrogenase B |
| 384 | U07643 | 105938 | LTF | lactotransferrin |
| 385 | M13452 | 377973 | LMNA | lamin A/C |
| 386 | D37766 | 75517 | LAMB3 | laminin, beta 3 |
| 387 | L13210 | 79339 | LGALS3BP | lectin, galactoside-binding, soluble, 3 binding protein |
| 388 | AF002672 | 152944 | LOH11CR2A | loss of heterozygosity, 11, chromosomal region 2, gene A |
| 389 | BE621666 | 296398 | LAPTM4B | lysosomal associated protein transmembrane 4 beta |
| 390 | L08895 | 78995 | MEF2C | MADS box transcription enhancer factor 2, polypeptide C (myocyte enhancer factor 2C) |
| 391 | AA779709 | 7457 | MAGE-E1 | MAGE-E1 protein |

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|---|---|---|---|---|
| 392 | M33906 | 198253 | HLA-DQA1 | major histocompatibility complex, class II, DQ alpha 1 |
| 393 | AW298180 | 2256 | MMP7 | matrix metalloproteinase 7 (matrilysin, uterine) |
| 394 | AF017418 | 104105 | MEIS2 | Meis1, myeloid ecotropic viral integration site 1 homolog 2 (mouse) |
| 395 | J02854 | 9615 | MYL9 | myosin, light polypeptide 9, regulatory |
| 396 | AF203032 | 198760 | NEFH | neurofilament, heavy polypeptide 200kDa |
| 397 | M12267 | 75485 | OAT | ornithine aminotransferase (gyrate atrophy) |
| 398 | U90878 | 75807 | PDLIM1 | PDZ and LIM domain 1 (elfin) |
| 399 | M22430 | 76422 | PLA2G2A | phospholipase A2, group IIA (platelets, synovial fluid) |
| 400 | D00244 | 77274 | PLAU | plasminogen activator, urokinase |
| 401 | AL045876 | 430637 | PTGDS | prostaglandin D2 synthase 21kDa (brain) |
| 402 | AF043498 | 423634 | PSCA | prostate stem cell antigen |
| 403 | NM_006394 | 278503 | RIG | regulated in glioma |
| 404 | NM_003979 | 194691 | RAI3 | retinoic acid induced 3 |
| 405 | AA173755 | 301198 | ROBO1 | roundabout, axon guidance receptor, homolog 1 (Drosophila) |
| 406 | AW965789 | 66450 | SENP1 | sentrin/SUMO-specific protease |
| 407 | M93056 | 183583 | SERPINB1 | serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1 |
| 408 | M13690 | 151242 | SERPING1 | serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1 |
| 409 | W73992 | 132792 | SDCCAG43 | serologically defined colon cancer antigen 43 |
| 410 | X51441 | 332053 | SAA1 | serum amyloid A1 |
| 411 | NM_006456 | 288215 | STHM | sialyltransferase |
| 412 | AF215636 | 5944 | SLC11A3 | solute carrier family 11 (proton-coupled divalent metal ion transporters), member 3 |
| 413 | U59299 | 90911 | SLC16A5 | solute carrier family 16 (monocarboxylic acid transporters), member 5 |
| 414 | M55531 | 33084 | SLC2A5 | solute carrier family 2 (facilitated glucose/fructose transporter), member 5 |
| 415 | M81635 | 160483 | STOM | stomatin |

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|---|---|---|---|---|
| 416 | BF514189 | 345728 | SOCS3 | suppressor of cytokine signaling 3 |
| 417 | AI423028 | 71622 | SMARCD3 | SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3 |
| 418 | AK001617 | 24948 | SNCAIP | synuclein, alpha interacting protein (synphilin) |
| 419 | U21847 | 82173 | TIEG | TGFB inducible early growth response |
| 420 | M12670 | 5831 | TIMP1 | tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor) |
| 421 | U54831 | 75248 | TOP2B | topoisomerase (DNA) II beta 180kDa |
| 422 | NM_003241 | 2387 | TGM4 | transglutaminase 4 (prostate) |
| 423 | W72411 | 137569 | TP73L | tumor protein p73-like |
| 424 | D88154 | 103665 | VILL | villin-like |
| 425 | X63187 | 2719 | WFDC2 | WAP four-disulfide core domain 2 |
| 426 | AF122922 | 284122 | WIF1 | WNT inhibitory factor 1 |
| 427 | AA916688 | 85155 | ZFP36L1 | zinc finger protein 36, C3H type-like 1 |
| 428 | BF055342 | 326801 | ZNF6 | zinc finger protein 6 (CMPX1) |
| | **function unknown** | | | |
| 429 | AA706316 | 32343 | ZD52F10 | hypothetical gene ZD52F10 |
| 430 | U57961 | 181304 | 13CDNA73 | hypothetical protein CG003 |
| 431 | AA709155 | 104800 | FLJ10134 | hypothetical protein FLJ10134 |
| 432 | AK001021 | 22505 | FLJ10159 | hypothetical protein FLJ10159 |
| 433 | AA180145 | 351270 | LOC152485 | hypothetical protein LOC152485 |
| 434 | AA133590 | 377830 | MGC44669 | hypothetical protein MGC44669 |
| 435 | NM_014766 | 75137 | KIAA0193 | KIAA0193 gene product |
| 436 | AI741882 | 278436 | KIAA1474 | KIAA1474 protein |
| 437 | BF431643 | 15420 | KIAA1500 | KIAA1500 protein |
| 438 | N62352 | 24790 | KIAA1573 | KIAA1573 protein |
| 439 | T78873 | 9587 | KIAA2002 | KIAA2002 protein |
| 440 | AK022877 | 49476 | | Homo sapiens cDNA FLJ12815 fis, clone NT2RP2002546. |
| 441 | AI566720 | 380045 | | Homo sapiens cDNA FLJ34528 fis, clone HLUNG2008066. |
| 442 | BE885999 | 397414 | | Homo sapiens cDNA: FLJ20860 fis, clone ADKA01632. |
| 443 | AK025909 | 288741 | | Homo sapiens cDNA: FLJ22256 fis, clone HRC02860. |
| 444 | AI269367 | 101307 | | Homo sapiens HUT11 protein mRNA, |

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|---|---|---|---|---|
| | | | | partial 3' UTR |
| 445 | AL050204 | 28540 | | Homo sapiens mRNA; cDNA DKFZp586F1223 (from clone DKFZp586F1223) |
| 446 | AV733210 | 367688 | | Homo sapiens, clone IMAGE:4794726, mRNA |
| 447 | AI027791 | 132296 | | ESTs |
| 448 | BF111819 | 21470 | | ESTs |
| 449 | AA632025 | 444752 | | ESTs |
| 450 | BE868254 | 380149 | | ESTs |
| 451 | AW510657 | 156044 | | ESTs |
| 452 | AA620628 | 186486 | | ESTs |
| 453 | AI769569 | 112472 | | ESTs |
| 454 | T79422 | 119237 | | ESTs |
| 455 | AI052358 | 131741 | | ESTs |
| 456 | N95414 | 55168 | | ESTs, Weakly similar to neuronal thread protein [Homo sapiens] [H.sapiens] |
| 457 | BG163478 | 405950 | | ESTs, Weakly similar to BAI1_HUMAN Brain-specific angiogenesis inhibitor 1 precursor [H.sapiens] |

Table 7 Up-regulated genes in 10 PINs

| PRC Assignment | Accession No | Hs. | Symbol | Title |
|---|---|---|---|---|
| | **function known** | | | |
| 458 | BE466450 | 50628 | AP4S1 | adaptor-related protein complex 4, sigma 1 subunit |
| 459 | AW612403 | 293970 | ALDH6A1 | aldehyde dehydrogenase 6 family, member A1 |
| 460 | AJ130733 | 128749 | AMACR | alpha-methylacyl-CoA racemase |
| 461 | NM_001642 | 279518 | APLP2 | amyloid beta (A4) precursor-like protein 2 |
| 462 | X59066 | 405985 | ATP5A1 | ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1 |
| 463 | AF071202 | 139336 | ABCC4 | ATP-binding cassette, sub-family C (CFTR/MRP), member 4 |
| 464 | AB019038 | 44592 | HMT-1 | beta-1,4 mannosyltransferase |
| 465 | AF231023 | 55173 | CELSR3 | cadherin, EGF LAG seven-pass G-type |

| | | | | |
|---|---|---|---|---|
| | | | | receptor 3 (flamingo homolog, Drosophila) |
| 466 | AI817172 | 29423 | COLEC12 | collectin sub-family member 12 |
| 467 | Z21488 | 143434 | CNTN1 | contactin 1 |
| 468 | AF255443 | 268281 | CRNKL1 | Crn, crooked neck-like 1 (Drosophila) |
| 469 | NM_005436 | 288862 | D10S170 | DNA segment on chromosome 10 (unique) 170 |
| 470 | AI697792 | 21189 | DNAJA2 | DnaJ (Hsp40) homolog, subfamily A, member 2 |
| 471 | AF039918 | 80975 | ENTPD5 | ectonucleoside triphosphate diphosphohydrolase 5 |
| 472 | AF176699 | 49526 | FBXL4 | F-box and leucine-rich repeat protein 4 |
| 473 | M99487 | 1915 | FOLH1 | folate hydrolase (prostate-specific membrane antigen) 1 |
| 474 | NM_000159 | 184141 | GCDH | glutaryl-Coenzyme A dehydrogenase |
| 475 | AW967035 | 159572 | HS3ST3B1 | heparan sulfate (glucosamine) 3-O-sulfotransferase 3B1 |
| 476 | NM_005333 | 211571 | HCCS | holocytochrome c synthase (cytochrome c heme-lyase) |
| 477 | U26726 | 1376 | HSD11B2 | hydroxysteroid (11-beta) dehydrogenase 2 |
| 478 | U89281 | 11958 | RODH | 3-hydroxysteroid epimerase |
| 479 | U42408 | 18141 | LAD1 | ladinin 1 |
| 480 | L25931 | 152931 | LBR | lamin B receptor |
| 481 | Z30137 | 49998 | LDB3 | LIM domain binding 3 |
| 482 | AF001174 | 57732 | MAPK11 | mitogen-activated protein kinase 11 |
| 483 | M92449 | 264330 | ASAHL | N-acylsphingosine amidohydrolase (acid ceramidase)-like |
| 484 | W23499 | 118654 | ASAH2 | N-acylsphingosine amidohydrolase (non-lysosomal ceramidase) 2 |
| 485 | R22536 | 220324 | FLJ13052 | NAD kinase |
| 486 | AA704060 | 8248 | NDUFS1 | NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75kDa (NADH-coenzyme Q reductase) |
| 487 | AI805082 | 303171 | OR51E2 | olfactory receptor, family 51, subfamily E, member 2 |
| 488 | AK025460 | 286049 | PSA | phosphoserine aminotransferase |
| 489 | NM_021200 | 380812 | PLEKHB1 | pleckstrin homology domain containing, family B (evectins) member 1 |
| 490 | AI346354 | 75871 | PRKCBP1 | protein kinase C binding protein 1 |

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|---|---|---|---|---|
| 491 | AF044588 | 344037 | PRC1 | protein regulator of cytokinesis 1 |
| 492 | NM_012342 | 78776 | NMA | putative transmembrane protein |
| 493 | AL041152 | 13264 | RC3 | rabconnectin-3 |
| 494 | L10333 | 99947 | RTN1 | reticulon 1 |
| 495 | M32313 | 552 | SRD5A1 | steroid-5-alpha-reductase, alpha polypeptide 1 |
| 496 | U04735 | 352341 | STCH | stress 70 protein chaperone, microsome-associated, 60kDa |
| 497 | U66035 | 125565 | TIMM8A | translocase of inner mitochondrial membrane 8 homolog A (yeast) |
| 498 | AA907673 | 432605 | UGCG | UDP-glucose ceramide glucosyltransferase |
| 499 | AA164237 | 279840 | ZNF222 | zinc finger protein 222 |
| 500 | NM_006300 | 193583 | ZNF230 | zinc finger protein 230 |
| | **function unknown** | | | |
| 501 | AK023414 | 22972 | FLJ13352 | hypothetical protein FLJ13352 |
| 502 | AI341472 | 274337 | FLJ20666 | hypothetical protein FLJ20666 |
| 503 | N48613 | 311163 | FLJ30162 | hypothetical protein FLJ30162 |
| 504 | BG179141 | 7962 | FLJ30525 | hypothetical protein FLJ30525 |
| 505 | AW971484 | 105069 | LOC148418 | hypothetical protein LOC148418 |
| 506 | AK000569 | 107444 | LOC90075 | hypothetical protein LOC90075 |
| 507 | D43948 | 76989 | KIAA0097 | KIAA0097 gene product |
| 508 | AB011085 | 301658 | KIAA0513 | KIAA0513 gene product |
| 509 | AB011127 | 43107 | KIAA0555 | KIAA0555 gene product |
| 510 | AI151160 | 155983 | KIAA0677 | KIAA0677 gene product |
| 511 | T55178 | 9846 | KIAA1040 | KIAA1040 protein |
| 512 | AI094513 | 21896 | KIAA1136 | KIAA1136 protein |
| 513 | AA206763 | 7991 | C20orf102 | chromosome 20 open reading frame 102 |
| 514 | AF131828 | 7961 | C9orf25 | chromosome 9 open reading frame 25 |
| 515 | AA825819 | 7535 | LOC55871 | LOC55871 |
| 516 | AW135763 | 6375 | HT010 | uncharacterized hypothalamus protein HT010 |
| 517 | AK025329 | 7158 | | DKFZP566H073 protein |
| 518 | AL390127 | 433788 | | Homo sapiens mRNA; cDNA DKFZp761P06121 |
| 519 | AI074176 | 31535 | | Homo sapiens, clone IMAGE:3460742, mRNA, partial cds |
| 520 | AI133467 | 95612 | | ESTs |
| 521 | BF514823 | 119065 | | ESTs |

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|---|---|---|---|---|
| 522 | AA897408 | 190065 | | ESTs |
| 523 | AI478401 | 104591 | | ESTs |
| 524 | AA430571 | 104881 | | ESTs |
| 525 | AA521342 | 101428 | | ESTs |
| 526 | N62332 | 102728 | | ESTs |
| 527 | H17800 | 438858 | | ESTs |
| 528 | AA826048 | 117887 | | ESTs |
| 529 | AA677094 | 117035 | | ESTs |
| 530 | AA682521 | 117261 | | ESTs |
| 531 | AI554006 | 112694 | | ESTs |
| 532 | AI004966 | 445098 | | ESTs |
| 533 | N52767 | 23406 | | EST |
| 534 | BF109251 | 353121 | | ESTs, Weakly similar to hypothetical protein FLJ20378 |
| 535 | AI700341 | 110406 | | ESTs, Weakly similar to hypothetical protein FLJ20489 |
| 536 | AA743154 | 373991 | | ESTs, Weakly similar to neuronal thread protein |
| 537 | AI352507 | 263600 | | ESTs, Weakly similar to RL17_HUMAN 60S ribosomal protein L17 (L23) |

Table 8 Down-regulated Genes in 10 PINs

| PRC Assignment | Accession No | Hs. | Symbol | Title |
|---|---|---|---|---|
| | **function known** | | | |
| 538 | K03000 | 76392 | ALDH1A1 | aldehyde dehydrogenase 1 family, member A1 |
| 539 | AF055024 | 153489 | ASB1 | ankyrin repeat and SOCS box-containing 1 |
| 540 | M81844 | 87268 | ANXA8 | annexin A8 |
| 541 | X82206 | 153961 | ACTR1A | ARP1 actin-related protein 1 homolog A, centractin alpha (yeast) |
| 542 | AW014316 | 1578 | BIRC5 | baculoviral IAP repeat-containing 5 (survivin) |
| 543 | S67310 | 69771 | BF | B-factor, properdin |
| 544 | AF132972 | 279772 | CGI-38 | brain specific protein |
| 545 | BE826171 | 100686 | BCMP11 | breast cancer membrane protein 11 |
| 546 | AF134640 | 7235 | CACNG3 | calcium channel, voltage-dependent, gamma subunit 3 |

| | | | | |
|---|---|---|---|---|
| 547 | AF177775 | 76688 | CES1 | carboxylesterase 1 (monocyte/macrophage serine esterase 1) |
| 548 | Z18951 | 74034 | CAV1 | caveolin 1, caveolae protein, 22kDa |
| 549 | K01144 | 84298 | CD74 | CD74 antigen |
| 550 | NM_002996 | 80420 | CX3CL1 | chemokine (C-X3-C motif) ligand 1 |
| 551 | U58514 | 154138 | CHI3L2 | chitinase 3-like 2 |
| 552 | W19536 | 363572 | CEPT1 | choline/ethanolaminephosphotransferase |
| 553 | U16306 | 81800 | CSPG2 | chondroitin sulfate proteoglycan 2 (versican) |
| 554 | AF101051 | 7327 | CLDN1 | claudin 1 |
| 555 | AI150272 | 258811 | COPG2 | coatomer protein complex, subunit gamma 2 |
| 556 | AV712344 | 285401 | CSF2RB | colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage) |
| 557 | NM_001733 | 1279 | C1R | complement component 1, r subcomponent |
| 558 | K02765 | 284394 | C3 | complement component 3 |
| 559 | AF081287 | 4076 | CTDP1 | CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) phosphatase, subunit 1 |
| 560 | L12579 | 147049 | CUTL1 | cut-like 1, CCAAT displacement protein (Drosophila) |
| 561 | D86977 | 78054 | DDX38 | DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 38 |
| 562 | M26602 | 274463 | DEFA1 | defensin, alpha 1, myeloid-related sequence |
| 563 | AF097021 | 273321 | GW112 | differentially expressed in hematopoietic lineages |
| 564 | NM_006182 | 71891 | DDR2 | discoidin domain receptor family, member 2 |
| 565 | NM_001953 | 73946 | ECGF1 | endothelial cell growth factor 1 (platelet-derived) |
| 566 | BF981201 | 408061 | FABP5 | fatty acid binding protein 5 (psoriasis-associated) |
| 567 | AF112152 | 11494 | FBLN5 | fibulin 5 |
| 568 | U60115 | 239069 | FHL1 | four and a half LIM domains 1 |
| 569 | NM_002083 | 2704 | GPX2 | glutathione peroxidase 2 (gastrointestinal) |
| 570 | NM_002084 | 386793 | GPX3 | glutathione peroxidase 3 (plasma) |

| | | | | |
|---|---|---|---|---|
| 571 | NM_002081 | 2699 | GPC1 | glypican 1 |
| 572 | AI887814 | 4953 | GOLGA3 | golgi autoantigen, golgin subfamily a, 3 |
| 573 | D21239 | 9195 | GRF2 | guanine nucleotide-releasing factor 2 (specific for crk proto-oncogene) |
| 574 | NM_006308 | 41707 | HSPB3 | heat shock 27kDa protein 3 |
| 575 | AK001601 | 69594 | HMG20A | high-mobility group 20A |
| 576 | J02770 | 36602 | IF | I factor (complement) |
| 577 | S81914 | 76095 | IER3 | immediate early response 3 |
| 578 | AI922295 | 413826 | IGHG3 | immunoglobulin heavy constant gamma 3 (G3m marker) |
| 579 | X67301 | 153261 | IGHM | immunoglobulin heavy constant mu |
| 580 | AW518944 | 76325 | IGJ | immunoglobulin J polypeptide, linker protein for immunoglobulin alpha and mu polypeptides |
| 581 | AK026991 | 61790 | IPO4 | importin 4 |
| 582 | M31159 | 77326 | IGFBP3 | insulin-like growth factor binding protein 3 |
| 583 | AK026736 | 57664 | ITGB6 | integrin, beta 6 |
| 584 | NM_002198 | 80645 | IRF1 | interferon regulatory factor 1 |
| 585 | U72882 | 50842 | IFI35 | interferon-induced protein 35 |
| 586 | M13143 | 1901 | KLKB1 | kallikrein B, plasma (Fletcher factor) 1 |
| 587 | U07643 | 105938 | LTF | lactotransferrin |
| 588 | AF025534 | 77062 | LILRB5 | leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 5 |
| 589 | AI563896 | 1569 | LHX2 | LIM homeobox protein 2 |
| 590 | AA644276 | 102267 | LOX | lysyl oxidase |
| 591 | M81141 | 73931 | HLA-DQB1 | major histocompatibility complex, class II, DQ beta 1 |
| 592 | BF697545 | 365706 | MGP | matrix Gla protein |
| 593 | NM_004530 | 111301 | MMP2 | matrix metalloproteinase 2 (gelatinase A) |
| 594 | AW298180 | 2256 | MMP7 | matrix metalloproteinase 7 (matrilysin) |
| 595 | NM_005928 | 3745 | MFGE8 | milk fat globule-EGF factor 8 protein |
| 596 | AI023878 | 406591 | MTIF3 | mitochondrial translational initiation factor 3 |
| 597 | J05581 | 89603 | MUC1 | mucin 1, transmembrane |

| | | | | |
|---|---|---|---|---|
| 598 | M94132 | 315 | MUC2 | mucin 2, intestinal/tracheal |
| 599 | AJ293659 | 12909 | MCOLN1 | mucolipin 1 |
| 600 | J02854 | 9615 | MYL9 | myosin, light polypeptide 9, regulatory |
| 601 | AB037787 | 26229 | NLGN2 | neuroligin 2 |
| 602 | NM_006169 | 364345 | NNMT | nicotinamide N-methyltransferase |
| 603 | S51033 | 79396 | MPG | N-methylpurine-DNA glycosylase |
| 604 | N35034 | 8121 | NOTCH2 | Notch homolog 2 (Drosophila) |
| 605 | NM_006163 | 75643 | NFE2 | nuclear factor (erythroid-derived 2), 45kDa |
| 606 | AW949776 | 3187 | NFX1 | nuclear transcription factor, X-box binding 1 |
| 607 | M13692 | 572 | ORM1 | orosomucoid 1 |
| 608 | BF115519 | 14125 | PA26 | p53 regulated PA26 nuclear protein |
| 609 | L03203 | 103724 | PMP22 | peripheral myelin protein 22 |
| 610 | AA398096 | 198278 | PFKFB4 | 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4 |
| 611 | AI660921 | 107125 | PLVAP | plasmalemma vesicle associated protein |
| 612 | D29833 | 2207 | PROL3 | proline rich 3 |
| 613 | N26005 | 303090 | PPP1R3C | protein phosphatase 1, regulatory (inhibitor) subunit 3C |
| 614 | BF673741 | 71119 | N33 | Putative prostate cancer tumor suppressor |
| 615 | AI004873 | 198281 | PKM2 | pyruvate kinase, muscle |
| 616 | H46145 | 27744 | RAB3A | RAB3A, member RAS oncogene family |
| 617 | AK026092 | 180040 | RIN3 | Ras and Rab interactor 3 |
| 618 | BG054844 | 6838 | ARHE | ras homolog gene family, member E |
| 619 | NM_003979 | 194691 | RAI3 | retinoic acid induced 3 |
| 620 | AA927661 | 201675 | RBM5 | RNA binding motif protein 5 |
| 621 | BF027943 | 2962 | S100P | S100 calcium binding protein P |
| 622 | AI719545 | 278431 | SCO2 | SCO cytochrome oxidase deficient homolog 2 (yeast) |
| 623 | X16150 | 82848 | SELL | selectin L (lymphocyte adhesion molecule 1) |
| 624 | J05176 | 234726 | SERPINA3 | serine (or cysteine) proteinase inhibitor, clade A, member 3 |
| 625 | BF126636 | 332053 | SAA1 | serum amyloid A1 |
| 626 | NM_004175 | 1575 | SNRPD3 | small nuclear ribonucleoprotein D3 polypeptide 18kDa |
| 627 | AF036109 | 193665 | SLC28A2 | solute carrier family 28 (sodium-coupled nucleoside transporter), member 2 |

| | | | | |
|---|---|---|---|---|
| 628 | AF058918 | 5699 | SEDLP | spondyloepiphyseal dysplasia, late, pseudogene |
| 629 | NM_000348 | 1989 | SRD5A2 | steroid-5-alpha-reductase, alpha polypeptide 2 |
| 630 | AF059203 | 20580 | SOAT2 | sterol O-acyltransferase 2 |
| 631 | AA853967 | 124574 | TAS1R1 | taste receptor, type 1, member 1 |
| 632 | AF082185 | 8375 | TRAF4 | TNF receptor-associated factor 4 |
| 633 | AI091425 | 9030 | TONDU | TONDU |
| 634 | AA682533 | 44269 | TRIPIN | tripin |
| 635 | AB025254 | 283761 | PCTAIRE2BP | tudor repeat associator with PCTAIRE 2 |
| 636 | D17517 | 301 | TYRO3 | TYRO3 protein tyrosine kinase |
| 637 | AF000993 | 13980 | UTX | ubiquitously transcribed tetratricopeptide repeat gene, X chromosome |
| 638 | AW574558 | 121102 | VNN2 | vanin 2 |
| 639 | H20162 | 2126 | VIPR2 | vasoactive intestinal peptide receptor 2 |
| 640 | BE382636 | 25960 | MYCN | v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian) |
| 641 | X63187 | 2719 | WFDC2 | WAP four-disulfide core domain 2 |
| | **function unknown** | | | |
| 642 | AI042017 | 23756 | C1orf13 | chromosome 1 open reading frame 13 |
| 643 | AA614050 | 267566 | C14orf58 | chromosome 14 open reading frame 58 |
| 644 | AK023453 | 334721 | FLJ13391 | hypothetical protein FLJ13391 |
| 645 | BE465676 | 353196 | FLJ14564 | hypothetical protein FLJ14564 |
| 646 | AK026924 | 105642 | FLJ21936 | hypothetical protein FLJ21936 |
| 647 | AW195243 | 108812 | FLJ22004 | hypothetical protein FLJ22004 |
| 648 | BF965831 | 135121 | FLJ22415 | hypothetical protein FLJ22415 |
| 649 | AK026486 | 118183 | FLJ22833 | hypothetical protein FLJ22833 |
| 650 | AW271223 | 5890 | FLJ23306 | hypothetical protein FLJ23306 |
| 651 | AI359551 | 22015 | FLJ90119 | hypothetical protein FLJ90119 |
| 652 | BG054529 | 206501 | LOC57228 | hypothetical protein from clone 643 |
| 653 | AI149729 | 120557 | LOC285286 | hypothetical protein LOC285286 |
| 654 | AI089621 | 22051 | MGC15548 | hypothetical protein MGC15548 |
| 655 | AW005320 | 236547 | MGC22916 | hypothetical protein MGC22916 |
| 656 | AI076840 | 40808 | MGC33926 | hypothetical protein MGC33926 |
| 657 | AW340131 | 56382 | FLJ32384 | hypothetical protein MGC39389 |
| 658 | AK025996 | 209614 | MGC4415 | hypothetical protein MGC4415 |
| 659 | AA827188 | 351605 | MGC45417 | hypothetical protein MGC45417 |
| 660 | H04833 | 6336 | KIAA0672 | KIAA0672 product |
| 661 | AB033103 | 6385 | KIAA1277 | KIAA1277 protein |
| 662 | BG054798 | 26204 | KIAA1295 | KIAA1295 protein |

| | | | | |
|---|---|---|---|---|
| 663 | AI694131 | 29002 | KIAA1706 | KIAA1706 protein |
| 664 | AL137345 | 298850 | KIAA1936 | KIAA1936 protein |
| 665 | AK025585 | 380169 | | DKFZp727A071 |
| 666 | AB037861 | 112184 | | DKFZP586J0619 protein |
| 667 | W58516 | 12396 | | Homo sapiens cDNA FLJ33095 fis, clone TRACH2000708. |
| 668 | AW967916 | 31944 | | Homo sapiens cDNA FLJ33236 fis, clone ASTRO2002571. |
| 669 | AF052090 | 106620 | | Homo sapiens clone 23950 mRNA sequence |
| 670 | AL110236 | 321022 | | Homo sapiens mRNA; cDNA DKFZp566P1124 (from clone DKFZp566P1124) |
| 671 | BE348293 | 29283 | | Homo sapiens proteoglycan link protein mRNA, complete cds. |
| 672 | AI139601 | 120590 | | Homo sapiens, clone IMAGE:5750475, mRNA |
| 673 | H42381 | 348805 | | hypothetical protein DKFZp667B0210 |
| 674 | AA180005 | 115029 | | ESTs |
| 675 | AA648546 | 230703 | | ESTs |
| 676 | AI916303 | 7444 | | ESTs |
| 677 | AA700898 | 113117 | | ESTs |
| 678 | AI246644 | 259679 | | ESTs |
| 679 | AI807279 | 443735 | | ESTs |
| 680 | AI160304 | 28313 | | ESTs |
| 681 | AA768888 | 446195 | | ESTs |
| 682 | BE502928 | 445376 | | ESTs |
| 683 | AA568515 | 293510 | | ESTs |
| 684 | AI732560 | 215976 | | ESTs |
| 685 | AI821961 | 126215 | | ESTs |
| 686 | AA928743 | 132527 | | ESTs |
| 687 | AA910771 | 130421 | | ESTs |
| 688 | AA938326 | 127167 | | ESTs |
| 689 | AA897581 | 445725 | | ESTs |
| 690 | AA004313 | 446619 | | ESTs, Highly similar to HIRA-interacting protein 3 |
| 691 | H21968 | 285520 | | ESTs, Moderately similar to hypothetical protein FLJ20489 |
| 692 | AI223250 | 131365 | | ESTs, Weakly similar to T31613 hypothetical protein Y50E8A.i - Caenorhabditis elegans] |

Table 2 Primer sequences for semi-quantitative RT-PCR experiments

| Symbol | Forward primer | SEQ.ID .NO. | Reverse primer | SEQ.ID .NO. |
|---|---|---|---|---|
| AMACR | 5'-TCATGATCTCCC TCTAAGCACAT-3' | 1 | 5'-TGTTGCTGTGTGTTG GGTATAAG-3' | 2 |
| HOXC6 | 5'-CCTGGGGGTCA TTATGGCATTTT-3' | 3 | 5'-TTCTCCTACTGGCTA AACAAACG-3' | 4 |
| POV1 | 5'-GGTGCCTCTTAT CTCCTTCT-3' | 5 | 5'-CTTCCCTTTTTATTTC CTCT-3' | 6 |
| ABHD2 | 5'-GTACTTGGCTTA AAAGCAACCAG-3' | 7 | 5'-CTCAGTGACCTGGAT CTGACCT-3' | 8 |
| C20ORF 102 | 5'-AACCACTTCTTG CGAGTCCTT-3' | 9 | 5'-TATTCAGGTTGGCTG GTAGTCAC-3' | 10 |
| β-actin | 5'-TTGGCTTGACTC AGGATTTA-3' | 11 | 5'-TGGACTTGGGAGAGG ACTGG-3' | 12 |

Industrial Applicability

The gene-expression analysis of PRC and PIN described herein, obtained through a combination of laser-capture dissection and genome-wide cDNA microarray, has identified specific genes as targets for cancer prevention and therapy. Based on the expression of a subset of these differentially expressed genes, the present invention provides a molecular diagnostic markers for identifying or detecting either or both of PRC and PIN.

The methods described herein are also useful in the identification of additional molecular targets for prevention, diagnosis and treatment of either or both of PRC and PIN. The data reported herein add to a comprehensive understanding of PRC, facilitate development of novel diagnostic strategies, and provide clues for identification of molecular targets for therapeutic drugs and preventative agents. Such information contributes to a more profound understanding of prostatic tumorigenesis, and provide indicators for developing novel strategies for diagnosis, treatment, and ultimately prevention of PRC.

All patents, patent applications, and publications cited herein are incorporated by reference in their entirety. Furthermore, while the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope of the invention.

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CLAIMS

1. A method of diagnosing either or both of PRC and PIN or a predisposition to developing either or both of PRC and PIN in a subject, comprising determining a level of expression of a PRC -associated gene in a patient derived biological sample, wherein an increase or decrease of said level compared to a normal control level of said gene indicates that said subject suffers from or is at risk of developing either or both of PRC and PIN.

2. The method of claim 1, wherein said PRC -associated gene is selected from the group consisting of PRC 1-88, wherein an increase in said level compared to a normal control level indicates said subject suffers from or is at risk of developing either or both of PRC and PIN.

3. The method of claim 2, wherein said increase is at least 10% greater than said normal control level.

4. The method of claim 1, wherein said PRC -associated gene is selected from the group consisting of PRC 89-295, wherein a decrease in said level compared to a normal control level indicates said subject suffers from or is at risk of developing either or both of PRC and PIN.

5. The method of claim 4, wherein said decrease is at least 10% lower than said normal control level.

6. The method of claim 1, wherein said method further comprises determining said level of expression of a plurality of PRC -associated genes.

7. The method of claim 1, wherein the expression level is determined by any one method select from group consisting of:
(a) detecting the mRNA of the PRC -associated genes,
(b) detecting the protein encoded by the PRC -associated genes, and
(c) detecting the biological activity of the protein encoded by the PRC -associated genes,

8. The method of claim 1, wherein said level of expression is determined by detecting hybridization of a PRC -associated gene probe to a gene transcript of said patient-

derived biological sample.

9. The method of claim 8, wherein said hybridization step is carried out on a DNA array.

10. The method of claim 1, wherein said biological sample comprises an epithelial cell.

11. The method of claim 1, wherein said biological sample comprises either or both of PRC and PIN cell.

12. The method of claim 8, wherein said biological sample comprises an epithelial cell from a PRC or PIN.

13. A PRC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PRC 1-295.

14. A PRC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PRC 1-88.

15. A PRC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PRC 89-295.

16. A method of screening for a compound for treating or preventing either or both of PRC and PIN, said method comprising the steps of:

a) contacting a test compound with a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-295;

b) detecting the binding activity between the polypeptide and the test compound; and

c) selecting a compound that binds to the polypeptide.

17. A method of screening for a compound for treating or preventing either or both of PRC and PIN , said method comprising the steps of:

a) contacting a candidate compound with a cell expressing one or more marker genes, wherein the one or more marker genes is selected from the group consisting of PRC 1-295; and

b) selecting a compound that reduces the expression level of one or more marker genes selected from the group consisting of PRC 1-88, or elevates the expression level of one or more marker genes selected from the group consisting of PRC 89-295.

18. A method of screening for a compound for treating or preventing either or both of PRC and PIN, said method comprising the steps of:

a) contacting a test compound with a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-295;

b) detecting the biological activity of the polypeptide of step (a); and

c) selecting a compound that suppresses the biological activity of the polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-88 in comparison with the biological activity detected in the absence of the test compound, or enhances the the biological activity of the polypeptide encoded by a nucleic acid selected from the group consisting of PRC 89-295 in comparison with the biological activity detected in the absence of the test compound.

19. The method of claim 17, wherein said cell comprises a either or both of PRC and PIN cell.

20. A method of screening for compound for treating or preventing either or both of PRC and PIN, said method comprising the steps of:

a) contacting a candidate compound with a cell into which a vector comprising the transcriptional regulatory region of one or more marker genes and a reporter gene that is expressed under the control of the transcriptional regulatory region has been introduced, wherein the one or more marker genes are selected from the group consisting of PRC 1-295

b) measuring the activity of said reporter gene; and

c) selecting a compound that reduces the expression level of said reporter gene when said marker gene is an up-regulated marker gene selected from the group consisting of PRC 1-88 or that enhances the expression level of said reporter gene when said marker gene is a down-regulated marker gene selected from the group consisting of PRC 89-295, as compared to a control.

21. A kit comprising a detection reagent which binds to two or more nucleic acid sequences selected from the group consisting of PRC 1-295.

22. An array comprising a nucleic acid which binds to two or more nucleic acid sequences selected from the group consisting of PRC 1-295.

23. A method of treating or preventing either or both of PRC and PIN in a subject

comprising the step of administering to said subject a compound that decreases the expression or activity of a polypeptide encoded by a gene selected from the group consisting of PRC 1-88.

24. A method of treating or preventing either or both of PRC and PIN in a subject comprising administering to said subject an antisense nucleic composition, said composition comprising a nucleotide sequence complementary to a coding sequence selected from the group consisting of PRC 1-88.

25. A method of treating or preventing either or both of PRC and PIN in a subject comprising administering to said subject a siRNA composition, wherein said composition reduces the expression of a nucleic acid sequence selected from the group consisting of PRC 1-88.

26. A method for treating or preventing either or both of PRC and PIN in a subject comprising the step of administering to said subject a pharmaceutically effective amount of an antibody or fragment thereof that binds to a protein encoded by any one gene selected from the group consisting of PRC 1-88.

27. A method of treating or preventing either or both of PRC and PIN in a subject comprising administering to said subject a vaccine comprising a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 1-88 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide.

28. A method of treating or preventing either or both of PRC and PIN in a subject comprising administering to said subject a compoud that increases the expression or activity of PRC 89-295

29. A method of treating or preventing either or both of PRC and PIN in a subject comprising administering to said subject a pharmaceutically effeotive amount of polynucleotide select from group consisting of PRC 89-295, or polypeptide encoded thereby.

30. A method for treating or preventing either or both of PRC and PIN in a subject, said method comprising the step of administering a compound that is obtained by the method according to any one of claims 16-20.

31. A composition for treating or preventing either or both of PRC and PIN, said composition comprising a pharmaceutically effective amount of an antisense polynucleotide or small interfering RNA against a polynucleotide select from group consisting of PRC 1-88 as an active ingredient, and a pharmaceutically acceptable carrier.

32. A composition for treating or preventing either or both of PRC and PIN, said composition comprising a pharmaceutically effective amount of an antibody or fragment thereof that binds to a protein encoded by any one gene selected from the group consisting of PRC 1-88 as an active ingredient, and a pharmaceutically acceptable carrier.

33. A composition for treating or preventing either or both of PRC and PIN, said composition comprising a pharmaceutically effective amount of polynucleotide select from group consisting of PRC 89-295, or polypeptide encoded thereby as an active ingredient, and a pharmaceutically acceptable carrier.

34. A composition for treating or preventing either or both of PRC and PIN, said composition comprising a pharmaceutically effective amount of the compound selected by the method of any one of claims 16-20 as an active ingredient, and a pharmaceutically acceptable carrier.

35. A method of diagnosing PRC or a predisposition to developing PRC in a subject, comprising determining a level of expression of a PRC -associated gene in a patient derived biological sample, wherein an increase or decrease of said level compared to a normal control level of said gene indicates that said subject suffers from or is at risk of developing PRC.

36. The method of claim 35, wherein said PRC -associated gene is selected from the group consisting of PRC 296-321, wherein an increase in said level compared to a normal control level indicates said subject suffers from or is at risk of developing PRC.

37. The method of claim 36, wherein said increase is at least 10% greater than said normal control level.

38. The method of claim 35, wherein said PRC -associated gene is selected from the

group consisting of PRC 322-457, wherein a decrease in said level compared to a normal control level indicates said subject suffers from or is at risk of developing PRC.

39. The method of claim 38, wherein said decrease is at least 10% lower than said normal control level.

40. The method of claim 35, wherein said method further comprises determining said level of expression of a plurality of PRC -associated genes.

41. The method of claim 35, wherein the expression level is determined by any one method select from group consisting of:
(a) detecting the mRNA of the PRC -associated genes,
(b) detecting the protein encoded by the PRC -associated genes, and
(c) detecting the biological activity of the protein encoded by the PRC -associated genes,

42. The method of claim 35, wherein said level of expression is determined by detecting hybridization of a PRC -associated gene probe to a gene transcript of said patient-derived biological sample.

43. The method of claim 42, wherein said hybridization step is carried out on a DNA array.

44. The method of claim 35, wherein said biological sample comprises an epithelial cell.

45. The method of claim 35, wherein said biological sample comprises PRC cell.

46. The method of claim 42, wherein said biological sample comprises an epithelial cell from a PRC.

47. A PRC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PRC 296-457.

48. A PRC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PRC 296-321.

49. A PRC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PRC 322-457.

50. A method of screening for a compound for treating or preventing PRC, said method comprising the steps of:

a) contacting a test compound with a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 296-457;

b) detecting the binding activity between the polypeptide and the test compound; and

c) selecting a compound that binds to the polypeptide.

51. A method of screening for a compound for treating or preventing PRC, said method comprising the steps of:

a) contacting a candidate compound with a cell expressing one or more marker genes, wherein the one or more marker genes is selected from the group consisting of PRC 296-457; and

b) selecting a compound that reduces the expression level of one or more marker genes selected from the group consisting of PRC 296-321, or elevates the expression level of one or more marker genes selected from the group consisting of PRC 322-457.

52. A method of screening for a compound for treating or preventing PRC, said method comprising the steps of:

a) contacting a test compound with a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 296-457;

b) detecting the biological activity of the polypeptide of step (a); and

c) selecting a compound that suppresses the biological activity of the polypeptide encoded by a nucleic acid selected from the group consisting of PRC 296-321 in comparison with the biological activity detected in the absence of the test compound, or enhances the the biological activity of the polypeptide encoded by a nucleic acid selected from the group consisting of PRC 322-457 in comparison with the biological activity detected in the absence of the test compound.

53. The method of claim 51, wherein said cell comprises a PRC cell.

54. A method of screening for compound for treating or preventing PRC, said method comprising the steps of:

a) contacting a candidate compound with a cell into which a vector comprising the transcriptional regulatory region of one or more marker genes and a reporter gene that is expressed under the control of the transcriptional regulatory region has been introduced, wherein the one or more marker genes are selected from the group consisting of PRC 296-457

b) measuring the activity of said reporter gene; and

c) selecting a compound that reduces the expression level of said reporter gene when said marker gene is an up-regulated marker gene selected from the group consisting of PRC 296-321 or that enhances the expression level of said reporter gene when said marker gene is a down-regulated marker gene selected from the group consisting of PRC 322-457, as compared to a control.

55. A kit comprising a detection reagent which binds to two or more nucleic acid sequences selected from the group consisting of PRC 296-457.

56. An array comprising a nucleic acid which binds to two or more nucleic acid sequences selected from the group consisting of PRC 296-457.

57. A method of treating or preventing PRC in a subject comprising the step of administering to said subject a compound that decreases the expression or activity of a polypeptide encoded by a gene selected from the group consisting of PRC 296-321

58. A method of treating or preventing PRC in a subject comprising administering to said subject an antisense composition, said composition comprising a nucleotide sequence complementary to a coding sequence selected from the group consisting of PRC 296-321.

59. A method of treating or preventing PRC in a subject comprising administering to said subject a siRNA composition, wherein said composition reduces the expression of a nucleic acid sequence selected from the group consisting of PRC 296-321.

60. A method for treating or preventing PRC in a subject comprising the step of administering to said subject a pharmaceutically effective amount of an antibody or fragment thereof that binds to a protein encoded by any one gene selected from the group consisting of PRC 296-321.

61. A method of treating or preventing PRC in a subject comprising administering to said subject a vaccine comprising a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 296-321 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide.

62. A method of treating or preventing PRC in a subject comprising administering to said subject a compoud that increases the expression or activity of PRC 322-457

63. A method of treating or preventing PRC in a subject comprising administering to said subject a pharmaceutically effective amount of polynucleotide select from group consisting of PRC 322-457, or polypeptide encoded thereby.

64. A method for treating or preventing PRC in a subject, said method comprising the step of administering a compound that is obtained by the method according to any one of claims 50-54.

65. A composition for treating or preventing PRC, said composition comprising a pharmaceutically effective amount of an antisense polynucleotide or small interfering RNA against a polynucleotide select from group consisting of PRC 296-321 as an active ingredient, and a pharmaceutically acceptable carrier.

66. A composition for treating or preventing PRC, said composition comprising a pharmaceutically effective amount of an antibody or fragment thereof that binds to a protein encoded by any one gene selected from the group consisting of PRC 296-321 as an active ingredient, and a pharmaceutically acceptable carrier.

67. A composition for treating or preventing PRC, said composition comprising a pharmaceutically effective amount of polynucleotide select from group consisting of PRC 322-457, or polypeptide encoded thereby as an active ingredient, and a pharmaceutically acceptable carrier.

68. A composition for treating or preventing PRC, said composition comprising a pharmaceutically effective amount of the compound selected by the method of any one of claims 50-54 as an active ingredient, and a pharmaceutically acceptable carrier.

69. A method of diagnosing PIN or a predisposition to developing PIN in a subject, comprising determining a level of expression of a PRC -associated gene in a patient

derived biological sample, wherein an increase or decrease of said level compared to a normal control level of said gene indicates that said subject suffers from or is at risk of developing PIN.

70. The method of claim 69, wherein said PRC -associated gene is selected from the group consisting of PRC 458-537, wherein an increase in said level compared to a normal control level indicates said subject suffers from or is at risk of developing PIN.

71. The method of claim 70, wherein said increase is at least 10% greater than said normal control level.

72. The method of claim 69, wherein said PRC -associated gene is selected from the group consisting of PRC 538-692, wherein a decrease in said level compared to a normal control level indicates said subject suffers from or is at risk of developing PIN.

73. The method of claim 72, wherein said decrease is at least 10% lower than said normal control level.

74. The method of claim 69, wherein said method further comprises determining said level of expression of a plurality of PRC -associated genes.

75. The method of claim 69, wherein the expression level is determined by any one method select from group consisting of:
(a) detecting the mRNA of the PRC -associated genes,
(b) detecting the protein encoded by the PRC -associated genes, and
(c) detecting the biological activity of the protein encoded by the PRC -associated genes,

76. The method of claim 69, wherein said level of expression is determined by detecting hybridization of a PRC -associated gene probe to a gene transcript of said patient-derived biological sample.

77. The method of claim 76, wherein said hybridization step is carried out on a DNA array.

78. The method of claim 69, wherein said biological sample comprises an epithelial cell.

79. The method of claim 69, wherein said biological sample comprises PIN cell.

80. The method of claim 76, wherein said biological sample comprises an epithelial cell from a PIN.

81. A PRC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PRC 458-692.

82. A PRC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PRC 458-537.

83. A PRC reference expression profile, comprising a pattern of gene expression of two or more genes selected from the group consisting of PRC 538-692.

84. A method of screening for a compound for treating or preventing PIN or preventing PRC, said method comprising the steps of:

a) contacting a test compound with a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 458-692;

b) detecting the binding activity between the polypeptide and the test compound; and

c) selecting a compound that binds to the polypeptide.

85. A method of screening for a compound for treating or preventing PIN or preventing PRC, said method comprising the steps of:

a) contacting a candidate compound with a cell expressing one or more marker genes, wherein the one or more marker genes is selected from the group consisting of PRC 458-692; and

b) selecting a compound that reduces the expression level of one or more marker genes selected from the group consisting of PRC 458-537, or elevates the expression level of one or more marker genes selected from the group consisting of PRC 538-692.

86. A method of screening for a compound for treating or preventing PIN or preventing PRC, said method comprising the steps of:

a) contacting a test compound with a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 458-692;

b) detecting the biological activity of the polypeptide of step (a); and

c) selecting a compound that suppresses the biological activity of the polypeptide encoded by a nucleic acid selected from the group consisting of PRC 458-537 in comparison with the biological activity detected in the absence of the test compound, or enhances the the biological activity of the polypeptide encoded by a nucleic acid selected from the group consisting of PRC 538-692 in comparison with the biological activity detected in the absence of the test compound.

87. The method of claim 85, wherein said cell comprises a PIN cell.

88. A method of screening for compound for treating or preventing PIN, or preventing PRC, said method comprising the steps of:

a) contacting a candidate compound with a cell into which a vector comprising the transcriptional regulatory region of one or more marker genes and a reporter gene that is expressed under the control of the transcriptional regulatory region has been introduced, wherein the one or more marker genes are selected from the group consisting of PRC 458-692

b) measuring the activity of said reporter gene; and

c) selecting a compound that reduces the expression level of said reporter gene when said marker gene is an up-regulated marker gene selected from the group consisting of PRC 458-537 or that enhances the expression level of said reporter gene when said marker gene is a down-regulated marker gene selected from the group consisting of PRC 538-692, as compared to a control.

89. A kit comprising a detection reagent which binds to two or more nucleic acid sequences selected from the group consisting of PRC 458-692.

90. An array comprising a nucleic acid which binds to two or more nucleic acid sequences selected from the group consisting of PRC 458-692.

91. A method of treating or preventing PIN or preventing PRC in a subject comprising the step of administering to said subject a compound that decreases the expression or activity of a polypeptide encoded by a gene selected from the group consisting of PRC 458-537.

92. A method of treating or preventing PIN or preventing PRC in a subject comprising administering to said subject an antisense composition, said composition

comprising a nucleotide sequence complementary to a coding sequence selected from the group consisting of PRC 458-537.

93. A method of treating or preventing PIN or preventing PRC in a subject comprising administering to said subject a siRNA composition, wherein said composition reduces the expression of a nucleic acid sequence selected from the group consisting of PRC 458-537.

94. A method for treating or preventing PIN or preventing PRC in a subject comprising the step of administering to said subject a pharmaceutically effective amount of an antibody or fragment thereof that binds to a protein encoded by any one gene selected from the group consisting of PRC 458-537.

95. A method of treating or preventing PIN or preventing PRC in a subject comprising administering to said subject a vaccine comprising a polypeptide encoded by a nucleic acid selected from the group consisting of PRC 458-537 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide.

96. A method of treating or preventing PIN or preventing PRC in a subject comprising administering to said subject a compoud that increases the expression or activity of PRC 538-692

97. A method of treating or preventing PIN or preventing PRC in a subject comprising administering to said subject a pharmaceutically effective amount of polynucleotide select from group consisting of PRC 538-692, or polypeptide encoded thereby.

98. A method for treating or preventing PIN or preventing PRC in a subject, said method comprising the step of administering a compound that is obtained by the method according to any one of claims 84-88.

99. A composition for treating or preventing PIN or preventing PRC, said composition comprising a pharmaceutically effective amount of an antisense polynucleotide or small interfering RNA against a polynucleotide select from group consisting of PRC 458-537 as an active ingredient, and a pharmaceutically acceptable carrier.

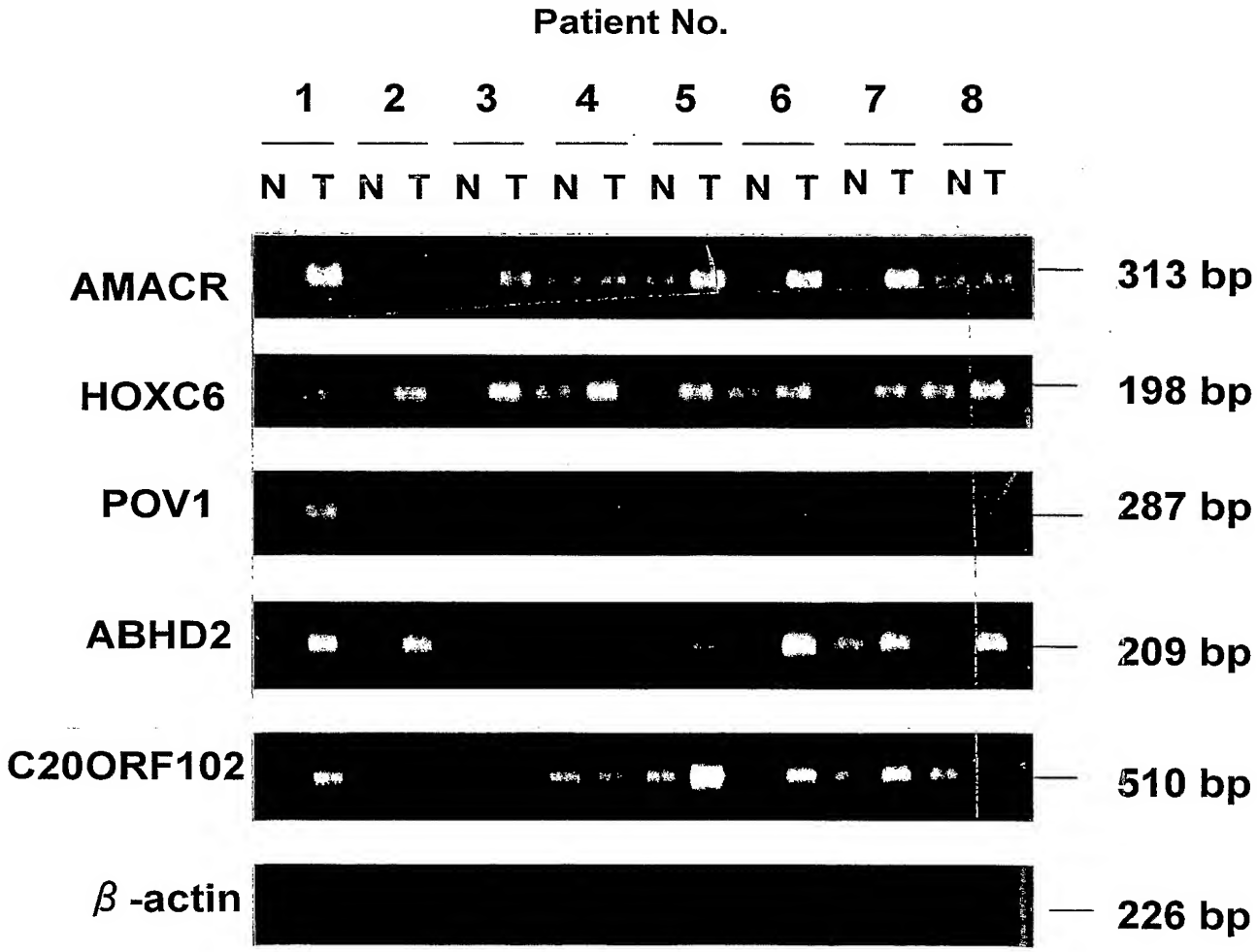
100. A composition for treating or preventing PIN or preventing PRC, said composition comprising a pharmaceutically effective amount of an antibody or fragment thereof

that binds to a protein encoded by any one gene selected from the group consisting of PRC 458-537 as an active ingredient, and a pharmaceutically acceptable carrier.

101. A composition for treating or preventing PIN or preventing PRC, said composition comprising a pharmaceutically effective amount of polynucleotide select from group consisting of PRC 538-692, or polypeptide encoded thereby as an active ingredient, and a pharmaceutically acceptable carrier.

102. A composition for treating or preventing PIN or preventing PRC, said composition comprising a pharmaceutically effective amount of the compound selected by the method of any one of claims 84-88 as an active ingredient, and a pharmaceutically acceptable carrier.

FIG.1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JAPAN AS REPRESENTED BY THE PRESIDENT OF THE UNIVERSITY OF TOKYO

<120> METHOD FOR DIAGNOSING PROSTATE CANCER

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